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# The global tendency in the research of biological activity in endophytic fungi: a scientometric analysis

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**Abstract**

Endophytic fungi colonize plants' interior tissues and organs, establishing a mutualistic relation without causing apparent harm. The biotechnological importance of these fungi has inspired research worldwide. Through scientometrics methods, it is possible to verify tendencies and gaps in different research area, collaborating with academic society to foster new studies and correlations. To identify the production of scientific knowledge relating to biological activity within endophytic fungi, we performed a survey on the platform Web of Science from studies published between 1997 and 2019 that have the following terms in the title, summary, or keywords: (Fung* AND Endophytic) AND (Bioprospecting OR Bio-prospecting OR Prospecting OR Bioactivit* OR "Biological Activit*"). A total of 458 studies were obtained, of which the following information was verified: publication year; average citations per year; journal; h-index of the journal; area of concentration; keyword and co-occurrence between them; network of collaboration between countries, institutions, and journals; bioassays realized and biological activity verified among endophytic fungi. China represents the nation-state that most frequently hosts research, followed by Brazil and India. According to our findings, the period between 2016 and 2019 showed an increase in the number of bioassays, nearly half of which (43%) proved to have antimicrobial activity, followed by anti-tumor activity (29%). These properties show that endophytic fungi can produce bioactive molecules of pharmaceutical and agronomic interest.

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## Introduction

Endophytic microbiota, including fungi, live asymptomatically in the living tissue of healthy plants, interacting with the host plant by aiding growth and resistance to herbivory, pathogens, and abiotic stresses (Rana et al. 2019a). In return, the endophytes receive protection and nutrients from the host (Rana et al. 2019b). However, at some point, this may turn pathogenic due to biotic or abiotic interferences (Rodriguez & Redman 2008), suggesting that the interaction between the fungus and the host plant should be characterized by a balance of potential of virulence of the fungus and sustained defense of the plant (Schulz et al. 2002, Saikkonen et al. 2004). In addition, it is known that every plant host can harbor an endophytic community. However, the frequency and composition of the endophytes depend on the environmental characteristics (mainly the climate), the geographic distribution of the plants, their age, and the specific tissue involved (Strobel 2003, Saikkonen et al. 2003).

Enzymes and secondary metabolites from endophytic fungi have shown tremendous biotechnological importance, principally in the composition of novel pharmaceuticals and the biological control of pests within agriculture (Gangadevi & Muthumary 2009a). Assays of bio-prospecting among these organisms verevealed natural composites with anti-bacterial, anti-fungal, anti-viral, anti-tumor, anti-inflammatory, anti-oxidant, and anti-protozoal activities with prospective for use in the treatment of disease and notable industrial applications (Strobel & Daisy 2003, Chapla et al. 2013). In addition, the manipulation of endophytic microbes enables more efficient methods of pharmaceutical production, minimizing the risk of extinction to plant species, some of which throughout the years have been suffering indiscriminate pressure from over harvest or malfeasance related to the extraction of their medicinal compounds (Peixoto et al. 2002).

The bibliographic survey realized by Jia et al. (2016) pointed out that 96 medicinal species of plants harbor endophytic fungi that produce bioactive compounds. The same hosts are commonly used as medication using direct consumption or extraction of active components. Species of the family Taxaceae are related to endophyte producers of taxol, a diterpenoid with anti-tumor activity mainly used to treat ovarian, uterine, and breast cancer (Gangadevi & Muthumary 2009b).

The endophytes possess a capacity to promote the accumulation of secondary metabolites within their host plants. Thus, researchers now isolate these microorganisms in plants with known therapeutic properties (Chen et al. 2016a). This is justified by the accelerated loss of medicinal species worldwide, compromising more than 50,000 species due to natural habitat destruction (Venieraki et al. 2017). To respond to the medical and agro-industrial demand, it is important to find alternative sources of bioactive compounds, especially for endangered and difficult to cultivate hosts (Atanasov et al. 2015).

Research involving the benefits of endophytic fungi has gained prominence throughout the world (Jalgaonwala et al. 2017), as presented by the review of Rashmi et al. (2019), which relates a list of more than 800 genera that present bio-active compounds, predominantly Alternaria, Aspergillus, *Colletotrichum*, *Fusarium*, *Penicillium* and *Phoma*. Intending to make a survey of the tendencies of biological activities of these microorganisms, scientometrics is an important ally in the evaluation of the quantitative aspects of research, including scientific journals, authors, and communication between the scientist (Macias-Chapula 1998, McGrath 1989). Considering the advancement of research verifying the biotechnological potential of endophytic fungi, in this review, we intend to contribute to the field with a scientometric analysis of global scientific literature on the biological activity of endophytic fungi.

## Materials & Methods

From the publications available on the platform Web of Science during the period of 1945 to 2019, a study was performed with the following search terms and respective Boolean operators' included in the title, summary, and/or keywords: (Fung* AND Endophytic) AND (Bioprospecting

OR Bio-prospecting OR Prospecting OR Bioactivit* OR "Biological Activit*"). The retrieved publications were individually evaluated for compliance with the theme and categorized into original correction and review articles.

For every paper recovered from the platform, the following information was registered: a) year of publication, b) average of citations of articles per year, c) journal in which the study was published, d) h-index of the journal, e) area of concentration, f) keywords and co-occurrence between them, g) authors and co-authors, h) most cited articles, i) h-index of the ten most published authors, j) national affiliation of the authors and co-authors, k) collaboration between countries, institutions, and journals, l) bio-assays realized per year and m) verified biological activities of endophytic fungi. The analysis was made in the software (R Core Team 2020) with the help of the package bibliometrix (Aria & Cuccurullo 2017). For the association between keywords, a co-occurrence network was produced, stemming from the walk trap clustering algorithm, which captures the structure of the community simulating random walks in the networks (Pons & Latapy 2006). A flow chart (Sankey diagram) presented the collaboration between countries, institutions, and journals.

## Results

The initial search resulted in 675 scientific publications, of which 458 correlated with the theme of the study, with 392 original articles, 3 corrections articles, and 63 review articles. The recovered publications are distributed among 197 journals. In addition, 1329 keywords and an average of 18.87 citations per article were found. Although the search began inin 1945, the first studies found approaching this theme date to 1997 (n = 2). There was a marked increase in studies from the year 2005. The highest number of studies was in 2018 (n = 70) (Fig. 1a). The largest average of citations per article was in 2006 (n = 159) (Fig. 1b). The significant number of citations in this year is due to the article "Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence" published in the Journal of Natural Products (Gunatilaka 2006), that received 528 citations, followed by the articles "Molecular characterization and anti-microbial activity of endophytic fungi from coffee plants" published in the World Journal of Microbiology and Biotechnology (Sette et al. 2006), with 62 citations, and "Marinamide, a novel alkaloid and its methyl ester produced by the application of mixed fermentation technique to two mangrove endophytic fungi from the South China Sea" published in the Chinese Science Bulletin (Zhu & Lin 2006), with 45 citations. This time series shows the visibility of articles in 2006, with experimental and review articles, bringing an ample variety and characterization of bioactive molecules. On the other hand, the articles published from 2011 do not achieve scientific repercussion sufficient to be cited.

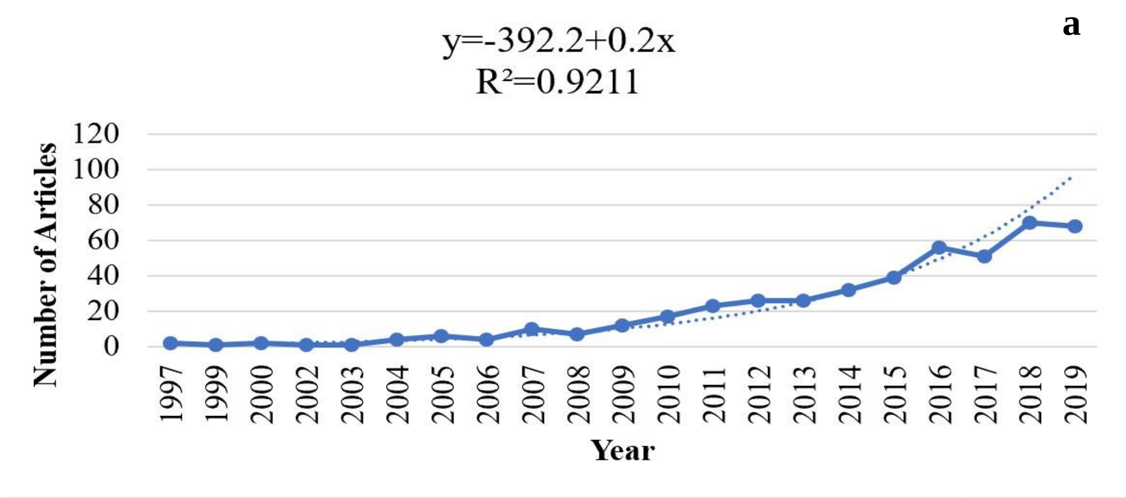
The journal Marine Drugs presented the highest number of publications (n = 16), followed by the World Journal of Microbiology & Biotechnology (n = 15) and the Journal of Natural Products (n = 15) (Fig. 2a). The journals with a higher h-index were the European Journal of Organic Chemistry (n = 13), followed by Marine Drug (n = 10), Planta Medica (n = 8), and Journal of Natural Products (n = 8) (Fig. 2b). Concerning the area of concentration, 52 studies are in the area of "Microbiology", followed by "Chemistry" (n = 45), and "Applied Biotechnology and Microbiology" (n = 43) (Fig. 2c). In addition, other areas of concentration were found (e.g. "Pharmacology and Pharmacy" "Bio-chemistry and Molecular Biology", and "Science and Technology"), noting that 229 articles fit into two or more areas of concentration.

The search for the most used keywords revealed that "natural-products" was included 95 times; followed by "metabolites" and "secondary metabolites", with 84 and 56 occurrences, respectively (Fig. 3a); both terms also appear prominently in the co-occurrence network (Fig. 3b). The keyword "natural-products" is used with the keyword's "metabolites," "identification", "diversity," "microorganisms", among others, as the keyword "secondary metabolites" is used with the keywords "biological-activity", "biological-activities", "endophytic fungus", "in-vitro" and "fungi", among others.

The authors and co-authors with the highest number of publications are Barbara Schulz (SCHULZ, B) and Siegfried Draeger (DRAEGER, S) (each with 16 articles), followed by Karsten

Krohn (KROHN, K) (n = 15) (Fig. 4a). The article with the most citations is “Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence” (Gunatilaka 2006) with 513 citations, the equivalent of 37 citations per year (Fig. 4b), which presents a review of microorganisms associated with plants as an enormous and unexplored resource of natural products, with chemical structures of biological relevance and a particular focus on endophytic fungi.

China represented the country with the highest number of publications (412 articles), followed by Brazil (n = 189) and India (n = 160) (Fig. 5a). The United States presents the largest collaboration network between countries, linking researchers in China, India, Brazil, Egypt, and the United Kingdom. The institutions most published between different journals are the University of São Paulo, Federal University of Paraná, and the Federal University of Minas Gerais (Brazil), and Sun Yat-sen University, China Agricultural University, and Zhejiang University (China). It was also verified that the University of Mississippi (USA) obtained the highest number of publications in different journals (n = 7). Sun Yat-sen University (China) was the only institution to publish Marine Drugs (Fig. 5b).



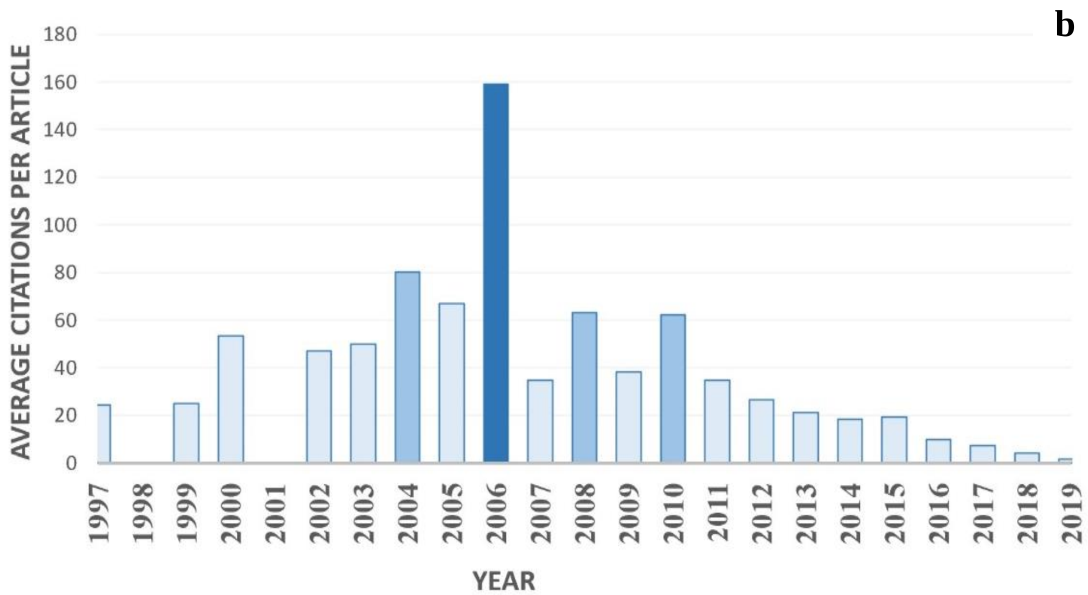




**Fig. 1** – Temporal distribution. (a) scientific production. (b) the average number of citations of articles per year. The exponential crescent curve (a) denotes a general tendency (p-value: 2.85-13).

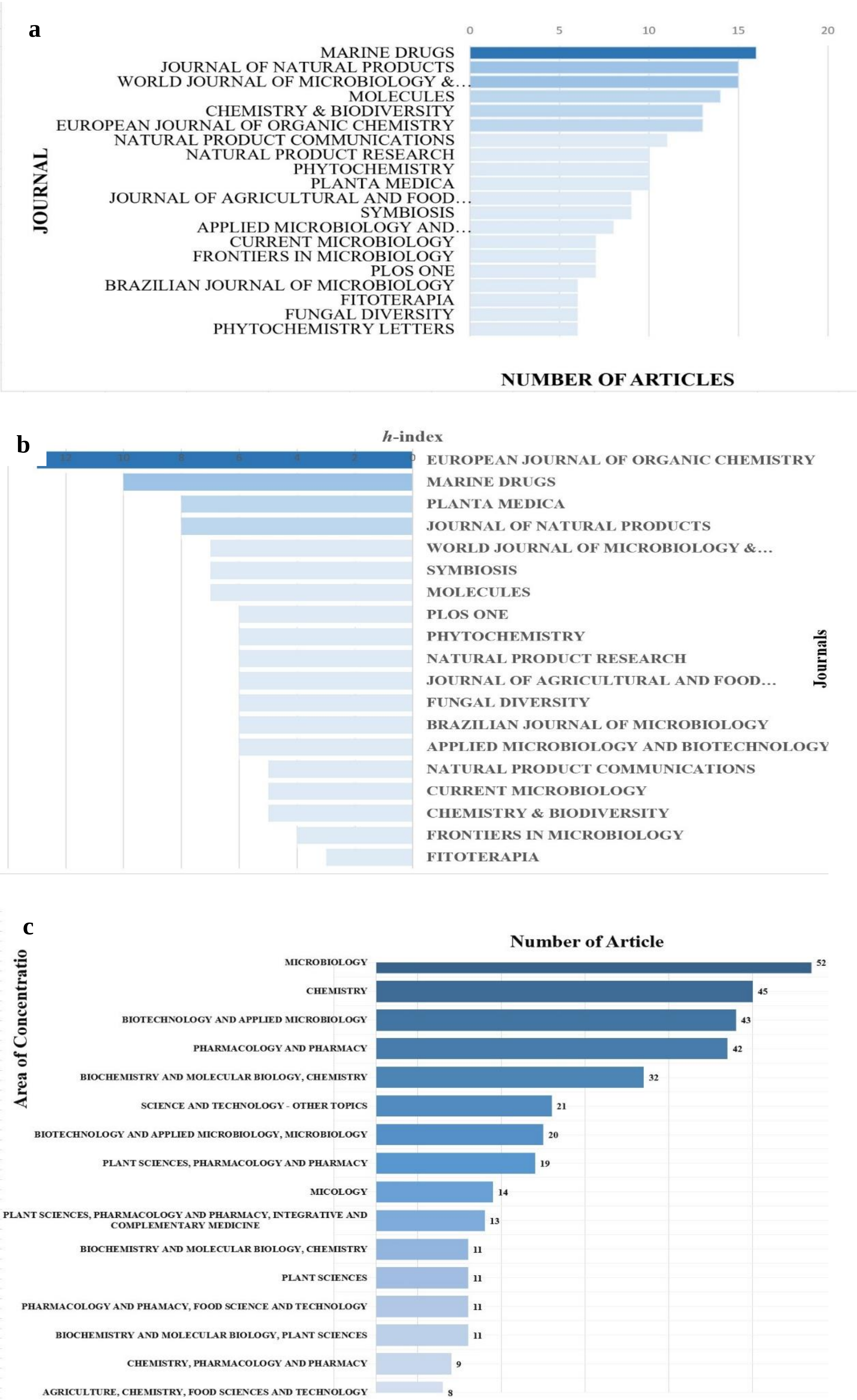



**Fig. 2** – Distribution of the (a) articles per journal, (b) journals per h-index, (c) articles per area of concentration.

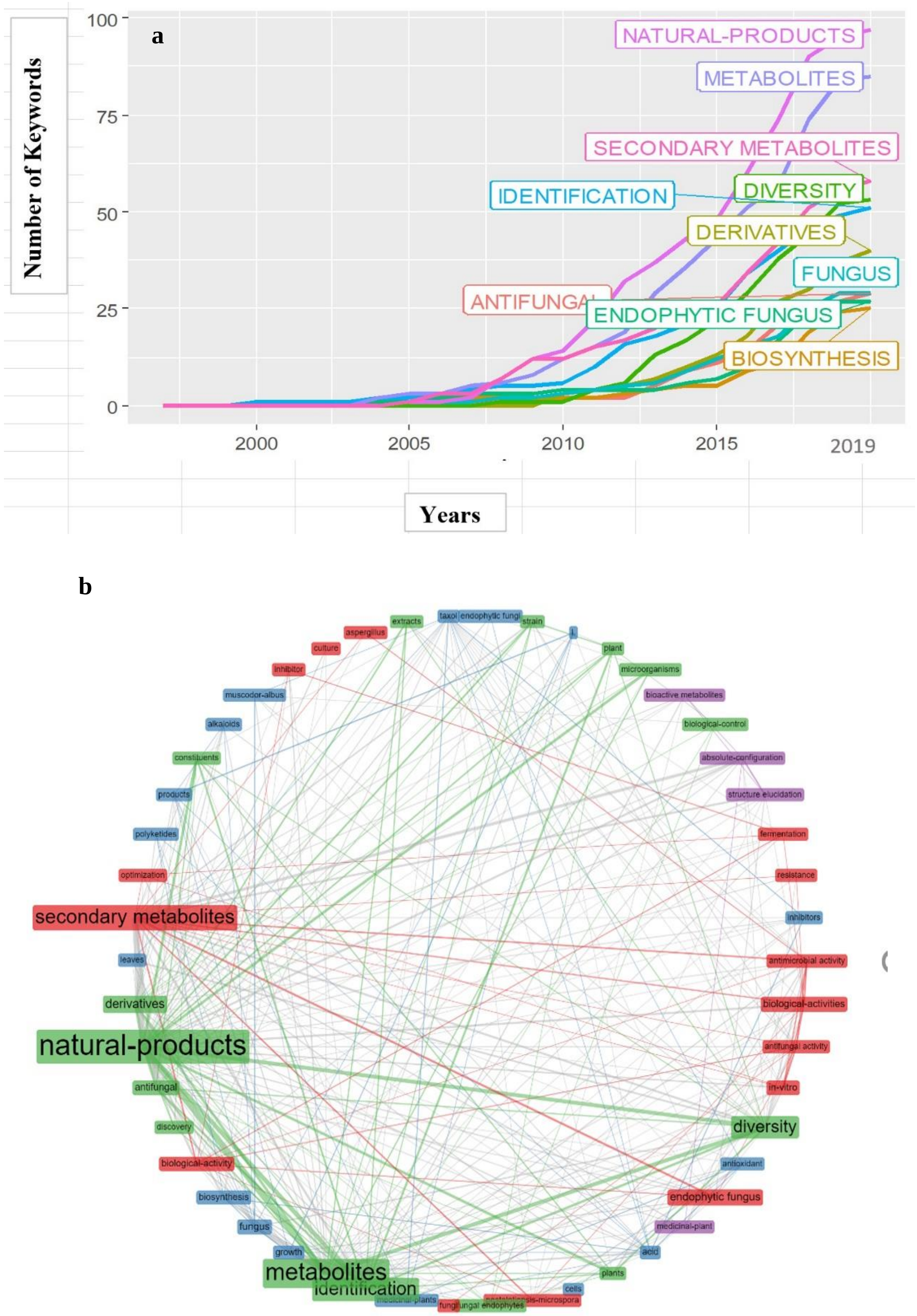



**Fig. 3** – Keywords found in the articles. a dynamics of the most frequent terms. b co-occurrence network among the most frequent terms.

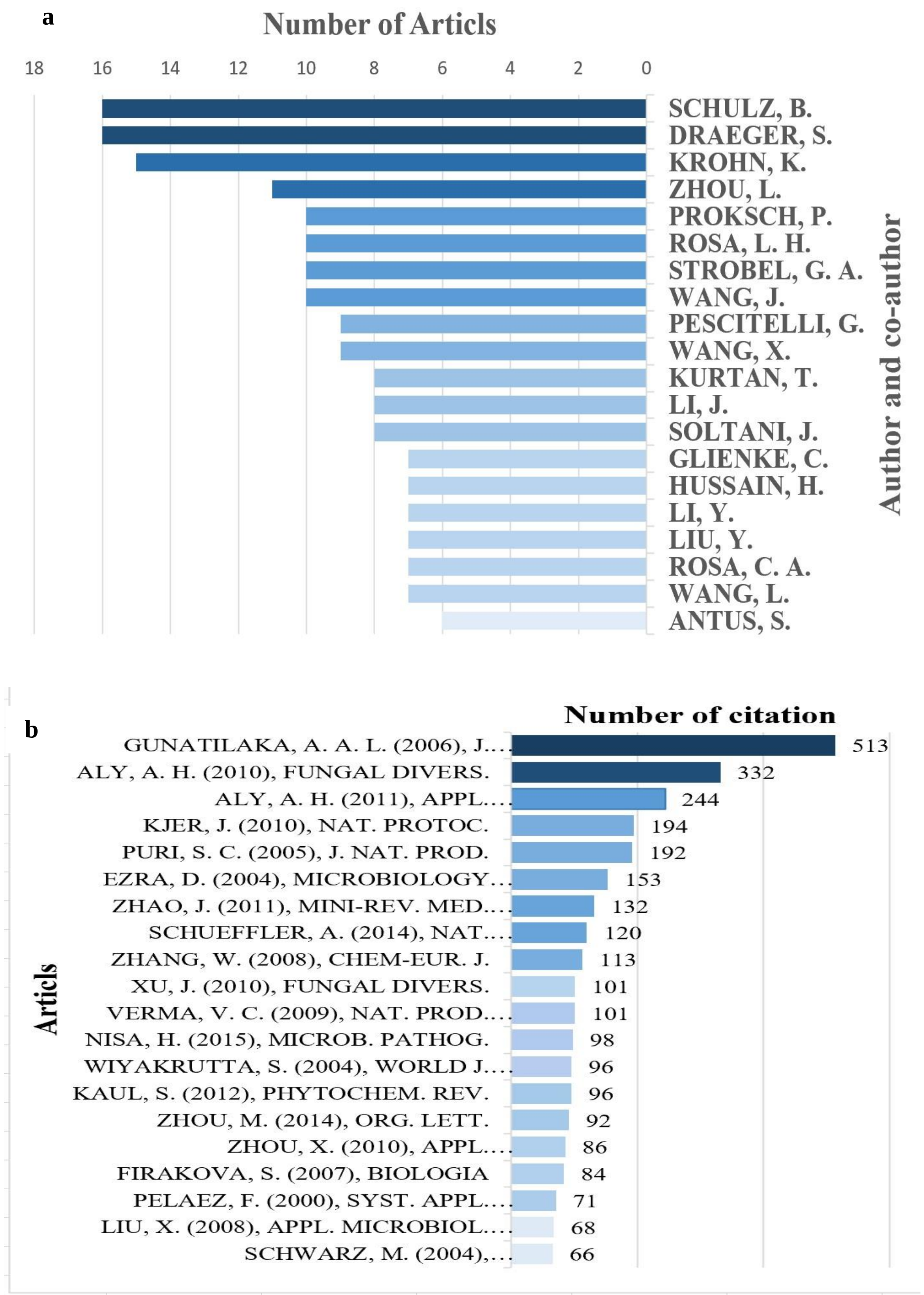



**Fig. 4** – Distribution of (a) authors following the total of publications, (b) articles following the total citations.

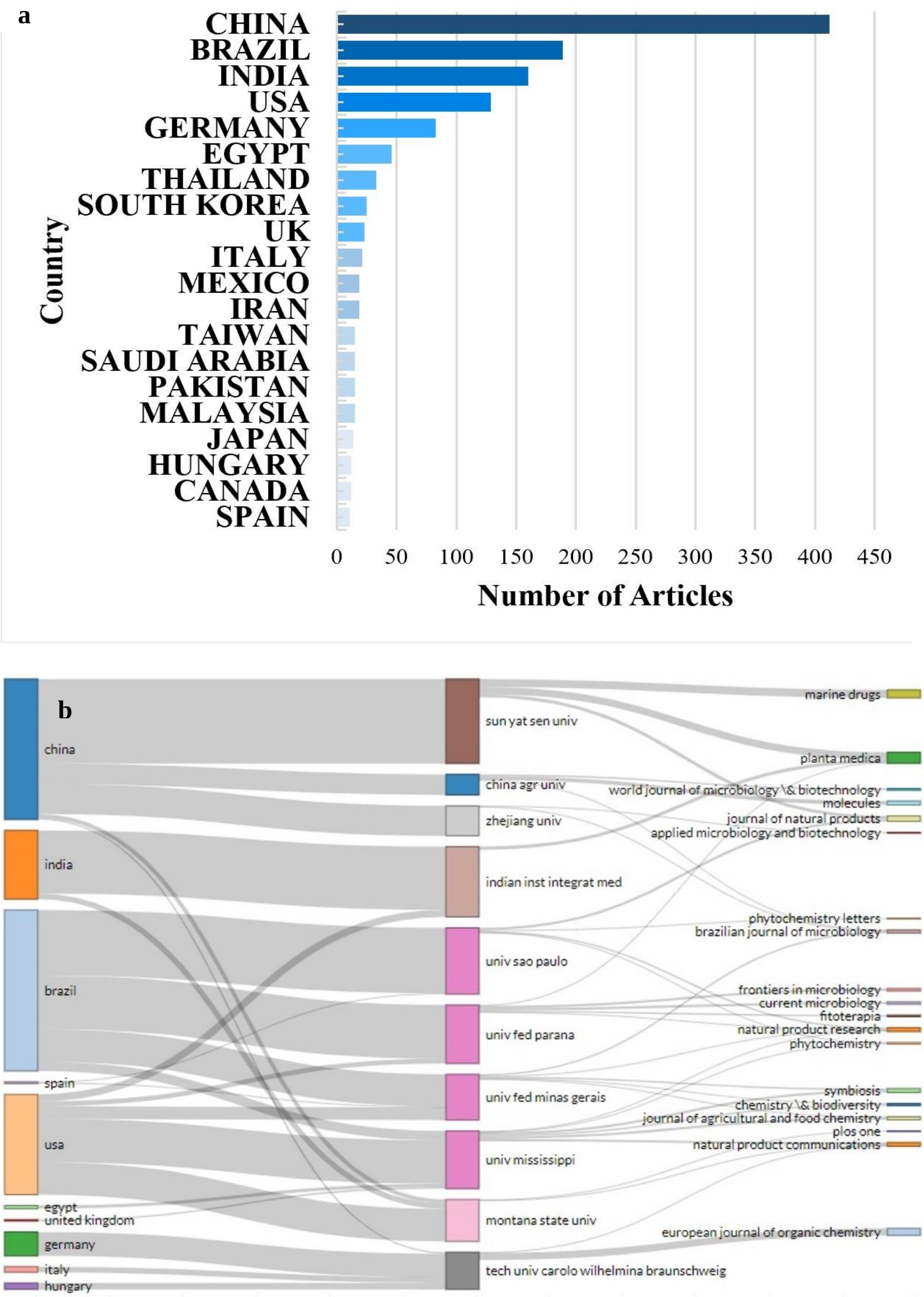



**Fig. 5** – Principal countries (a) to host research about the biological activity of endophytic fungi, (b) institutions and journals concerning collaboration of research.

The analyses related to biological activities addressed in the 395 experimental articles reveal 484 bio-assays (some studies verified only one activity and others more than one activity). A marked increase of bio-assays began in 2014, with the most in 2018 (n = 81), followed by 2019 (n = 79), 2016 (n = 51), 2017 (n = 48) e 2014 (n = 42) (Fig. 6).

The categorization of these biological activities resulted in 12 groups (e.g. antimicrobial, anti-tumor, enzymatic, anti-oxidant, insecticidal, anti-inflammatory, anti-diabetic, immunomodulatory, seed germination stimulant, anti-obesity, anti-algal, anti-hemolysis). The anti-microbial activity (anti-bacterial, anti-fungal, anti-parasitical, anti-viral) corresponded to almost half of the bio-assays (43%, n = 208), followed by anti-tumor (29%, n = 140) and enzymatic activity (9%, n = 44). Other types of activities (e.g. anti-oxidant, insecticidal, anti-inflammatory, anti-diabetic, immunomodulatory, seed germination stimulant, anti-obesity, anti-algal, anti-hemolysis) had less than 9% (Fig. 7).

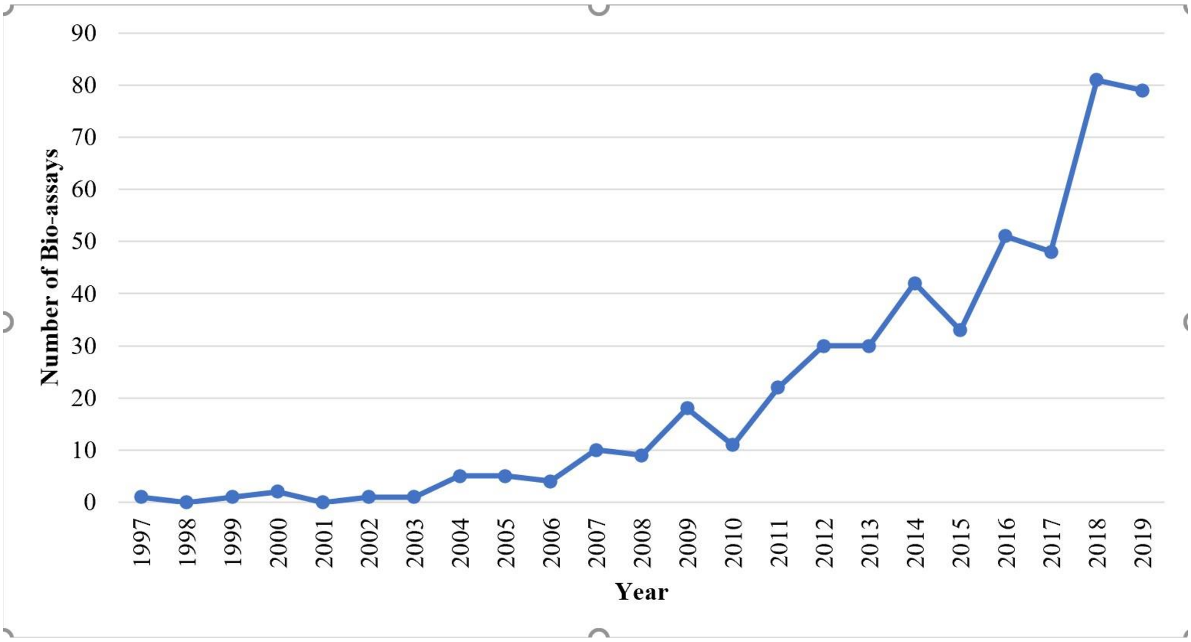



**Fig. 6** – Distribution of the bio-assays described in the scientific literature catalogued on the platform Web of Science, from 1997 to 2019.

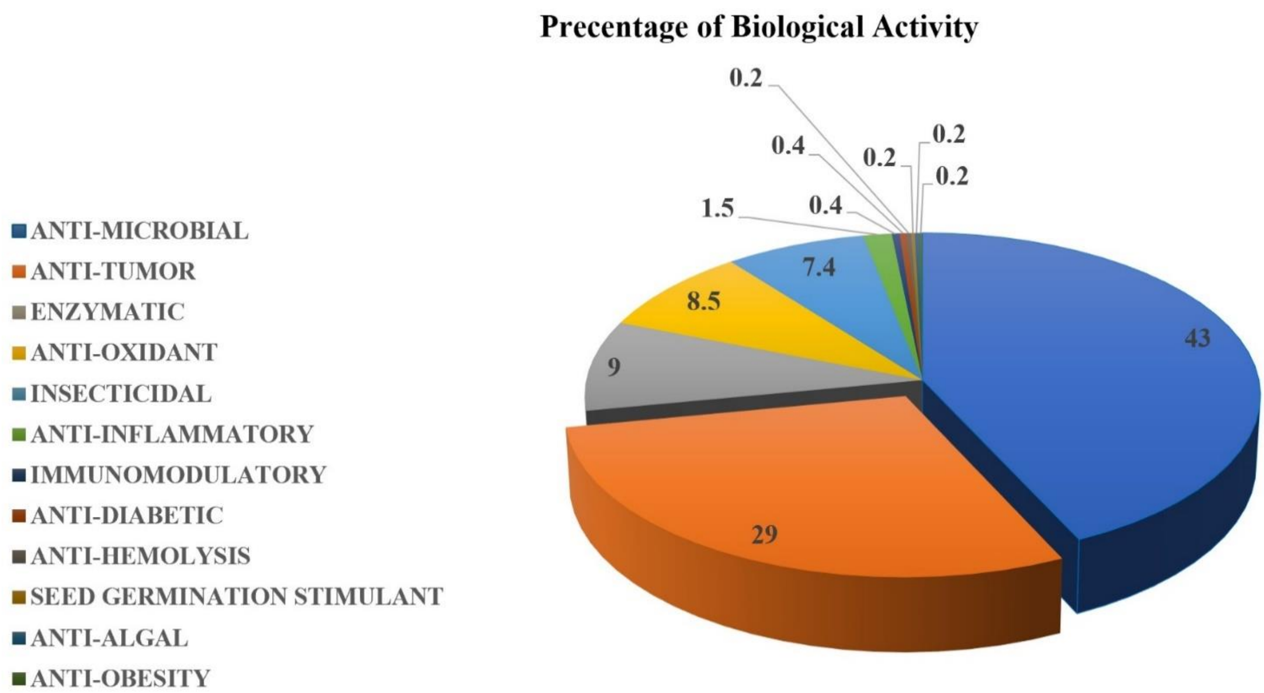



**Fig. 7** – Distribution of the articles following the type of biological activity.

## Discussion

Throughout the years, the evolution of publications about biological activity among endophytic fungi has increased researcher's interest in discovering novel bioactive compounds, considering that the number of scientific papers is the most efficient way to measure this growth (Verbeek et al. 2002). The first publications in this area began in 1997, thus corroborating with the survey of (Gupta et al. 2020), which mentions that the first experimental studies involving this theme, although few, date to the 1990's, therein marking the start and establishing appreciation amongst researchers for the bio-active compounds of these microorganisms. Although the first studies of the biotechnological importance of endophytic fungi were published just before the turn of the 21st century, these endophytes already had been related in previous studies (e.g. from 1866) and described in the fossilized tissue of plants, indicating that this mutualistic symbiotic relation is ancient (Redecker et al. 2000, Krings et al. 2007).

Since the discovery of the biotechnological potential of endophytes, a significant increase in scientific production in the area has been observed, beginning around the turn of the 2000s, which may be due to better financing amongst universities and research institutes throughout the world, offering grants for academic training and investment in science, in addition to the increased attention given for the newfound applicability in the treatment of illnesses (Frank & Meyer 2007). The increase of scientific research is due to the constant search for novel bioactive compounds with opportunities for application in medicine. Pandey et al. (2014) pointed out that these investigations will continue to advance, bringing better and more accurate perceptions of such compounds and their biological activities, a fact observed in this study that demonstrates a significant increase of scientific publications in 2018 and 2019.

The research involved in the biological activity of endophytic fungi is aligned in three large areas of concentration (microbiology [including applied microbiology], chemistry and biotechnology), because the metabolites and extracellular enzymes produced by these microorganisms can be applied in various industrial and agricultural processes (Yadav 2018). This encourages the isolation of endophytic fungi from hosts not yet studied in the search for new natural products and justifies the use of the keywords like "natural products", "metabolites," and "secondary metabolites." Strobel (2003) shows that the biological association between endophyte and host plant produces a diversity of secondary metabolites. This association may have developed a genetic system that permits transference between them. Meanwhile, because the publications were realized in a short and determined time (Fig. 1a) – less than two decades – the interaction between researchers can explain the repetition of methodologies, which facilitates the discussion of the data, and with this, the repetition of keywords (Chen et al. 2016b).

Between the ten authors with the most publications, four belong to institutions in Germany (Barbara Schulz, Siegfried Draeger, Karsten Krohn, and Peter Proksch). The German government has been establishing research and innovation in line with its growth policies, ensuring basic and essential conditions conducive to Education (Neves & Neves 2011). Within these policies, the 2005 program High tech Strategies stands out as the largest ongoing program of research and innovation; aside from this example, other policies can also be mentioned to foster the progress of research at universities (e.g. Initiative of Excellence, the Pact of Superior Education and the Pact for Research and Innovation) (Neves & Neves 2011).

However, in an evaluation between authors and co-authors, China holds the highest number of publications, pointing to investments in infrastructure and research financing, by public agencies and private companies. The National Science Foundation (2018), showed that since 2000 China increased its investments in research and development by an annual average of 18% compared to an average of 4% in the same period in the United States. This investment has already brought significant changes since 2018 when China surpassed the United States in the number of publications (Tollefson 2018), a result also observed in our study.

The data obtained in our study agree with those obtained by Gonçalves et al. (2017) demonstrated that China has the highest number of patents involving endophytic fungi applicable in pharmaceutical and cosmetic areas (31 patents, 86% of the documents found). Moreover, the Chinese territory is rich in biodiversity, with already more than 30,000 documented species of microorganisms, constituting a great natural potential and reservoir for scientific research in strategic sectors that may be of significant interest to the pharmaceutical and biotechnological industries. The goal of these industries is the development and commercialization of innovative medicines, principally for the treatment of chronic sicknesses and infections (Li 2010). Other developing countries, such as Brazil and India, appear in the ranking as those that most published findings verify biological activity among endophytic fungi and therefore also show interest in new research in this strategic area of development. Although Brazil holds second place amongst countries with the highest number of publications, Gonçalves et al. (2017) note that larger investments in the universities are still necessary for the furtherance of new technologies, with few registered patents yet related to endophytic fungi and their applications in the database of the National Institute of Industrial Property (INPI).

The journals that most published articles proving biological activity in endophytic fungi, Marine Drugs (3,5%), the World Journal of Microbiology & Biotechnology (3,3%) and the Journal of Natural Products (3,3%), are responsible for a little more than 10% of the publications that contribute to the biochemistry of endophytes, whether by marine or terrestrial hosts. With respect to the journal of the highest h-index, it was verified that the European Journal of Organic Chemistry, between 2005 and 2006 was already in 4th place in the ranking of this index (Bornmann et al. 2009), and thus presented an increase in the number of publications, becoming one of the 20 most relevant journals in the area of organic chemistry.

The United States predominantly orchestrates networks of research collaboration, which deserves to be highlighted, as it presents a disparity of technical and scientific knowledge concerning the bio-prospecting of fungi (Lima et al. 2018), which leads to an increase of cooperation and knowledge exchange with developing countries, notably China, India, and Brazil. The highest number of bio-assays occurred in 2018 (17%), 2019 (16%), 2016 (11%), and 2017 (10%), and were responsible for almost 55% of the total research, demonstrating a tendency of growth.

In almost half of the bio-assays, the active ingredients studied have shown more frequent antimicrobial properties; the clinical importance of pathogenic microorganisms, especially drug-resistant bacteria, justifies the search for substances with novel antimicrobial activities (Schmitt et al. 2011). Moreover, almost 30% of the bio-assays proved to have anti-tumor properties, bringing new perspectives for exploring drugs that fight cancer. Due to the great economic value of these therapies, Chen et al. (2014) relate that between 2010 and 2013, about 100 studies of secondary metabolites of endophytic fungi with these properties were in process, principally in China and India, and at least 30 novel metabolites were isolated.

## Conclusions

The analysis of the publications involving biological activity among endophytic fungi revealed a growing trend in the number of studies since the 2000s, with China, Brazil, and India representing the most research countries. In addition, the journals that most published scientific articles with results about bio-activity of endophytic fungi were Marine Drugs, the World Journal of Microbiology & Biotechnology, the Journal of Natural Products, and the European Journal of Organic Chemistry, the latter having the highest h-index. The rapid and important increase in the number of bio-assays between 2016 and 2019 points to the evolution of scientific production worldwide and reveals that developing countries have been concerned with seeking new sources of bioactive compounds within endophytic microorganisms. Studies that have proven anti-microbial and anti-tumor properties are highlighted, with the activities most frequently mentioned regarding the possibility of future applications.

## Author contribution

IRS, LLB, AMA, and SXS conceived of the presented idea, designed, directed the project wrote the manuscript with support from DDC and JPB. VKG, TAM, HHA, MAA, MMI, and AMA helped supervise the project. TAM, HHA, MMI and MAA lead the manuscript format, and all authors discussed the results and contributed to the final manuscript format.

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**Consent for publication**

All the authors have approved the manuscript for publication.

**Competing interests**

The authors declare no competing interests.

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