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RESEARCH ARTICLE

Aqueous extract of fresh leaves from *Alternanthera brasiliana* (L.) Kuntze: chemical evaluation and antimycobacterial and anticandidal activities

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Abstract

The focus of this study was to evaluate the presence and type of carbohydrates and phenolic molecules inaqueous extract (Aq-E) of *Alternanthera brasiliana* fresh leaves and its antioxidant and antimicrobial activities and cytotoxicity in vitro. The amount and types of the carbohydrates were measured by colorimetric and TLC methods. Phenolic compounds was detected by colorimetric assay and analysed by HPLC profiles. The antioxidant activity was evaluated by ABTS and Phosphomolybdenum methods. Antimicrobial activity was tested by microdilution method using microorganism models and antibiotic as positive control, the cytotoxicity in vitro was tested using *Artemia salina*. The results showed the presence of high amount of total sugars and uronic acids. TLC chromatograms showed mainly D-glucose, D-fructose, oligosaccharides and uronic acids in the Aq-E and a sugar alcohol in the methanolic extract. The colorimetric determination showed high concentration of phenolic compounds, which were visualised on the HPLC profiles, such as chemical markers of the Amaranthaceae family and several phenolic acids and flavonoids. The Aq-E demonstrated optimal antioxidant activities. The most important results were the excellent antibacterial and bactericidal activities against *Mycobacterium megmatis* (MIC = 15.6 µg/mL and MBC = 1000 µg/mL) and antimicrobial activity against *Candida albicans* (MIC = 31.2 µg/mL) and low cytotoxicity. Further possibilities for this plant extract will be to improve the pharmacological potential for developing new herbal medicines and possibly to study its association to allopathic antibiotics for prevention or treatment of infection diseases.

Keywords *Alternanthera brasiliana* · Amaranthaceae · Carbohydrates · *Mycobacterium smegmatis* · *Candida albicans* · Antioxidant · Antimicrobial

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Introduction

The scientific novelty of the present work is to show the concentration and types of the carbohydrates contained in aqueous extract from fresh leaves (Aq-E) of *Alternanthera brasiliana*, together with the phenolic compounds to amplify the biological activities of the extract, such as poly and oligosaccharides and uronic acids that also demonstrate antioxidant, antimicrobial and cicatrizing activities (Shevchuk et al. 2020; Bayar et al. 2016; Huang et al. 2012; Kanchana et al. 2013; Aoyagi et al. 2008). Previous studies (Zaier et al. 2020; Kumar et al. 2011) only report the nutritional composition in percentages of the fibre and carbohydrates of

the leaves, not in the leaf extract that contains the soluble carbohydrates. The current study intends to demonstrate the number of total sugars, reducing sugars, uronic acids and phenolics compounds in one fresh leaf of *A. brasiliana*. In addition, the aqueous extract was prepared using a simple process considering time and energy costs for industry, since most reports used previous leaf drying and powdering to prepare the hydroalcoholic or organic solvent extract, sometimes after an incubation period, which may not be feasible in industrial applications for further production of medicinal formulations. Furthermore, it is not easy to find previous reports about the antimicrobial activity of aqueous extract of *A. brasiliana* against *Mycobacterium smegmatis*.

Brazilian communities use the leaves of *Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae) in folk medicine, a branched perennial herb popularly known as "penicillin", "terramycin", "perpetuate of the bush" and "novalgina", to prepare teas or plasters to treat wounds and infection diseases. Several phytopharmacological activities of *A. brasiliana* extracts have been reported, mainly antioxidant, antimicrobial, anti-inflammatory, wound healing, antidematogenic and analgesic activities (Coutinho et al. 2018; Trapp et al. 2015; Pereira et al. 2013; Enechi et al. 2013; Formagio et al. 2012; Araújo and Onofre 2011; Kumar et al. 2011; Silva et al. 2011; Barua et al. 2009). However, the authors have been showed that the phenolic compounds, flavonoids and terpene derivatives are responsible for these biological activities, stimulating detailed studies about the amount, structure and biological effects of these compounds (Alencar-Filho et al. 2020; Hazli et al. 2019; Coutinho et al. 2018; Deladino et al. 2017; Trapp et al. 2015). There are several reports about the phenolic compounds, flavonoids and antibiotic compositions due to the presence of betacyanins, a purple pigment (Coutinho et al. 2018; Deladino et al. 2017; Khan and Giridhar 2015; Trapp et al. 2015).

However, the extent of climate diversity from the North to South of Brazil should be considered, leading to different soil compositions and spectral bands of the sunlight which can cause alterations in the morphology, biochemical metabolism and endophytic microorganisms of medicinal plants (Hrynkiewicz et al. 2019; Trapp et al. 2015; Macedo et al. 2014) and consequently the presence or absence and quantity of bioactive molecules in *A. brasiliana* extracts. During the development of the present study, differences were observed in the HPLC profiles of the *A. brasiliana* cultivated in the northeast, southeast and south region of Brazil (data not shown), clearly demonstrated that each of these plants contains different phytomolecules and pharmacological activities. In this way, HPLC profiles aim to show the geographical origin and authenticity of medicinal plants and can be used for quality control of the commercial herbal products (Shevchuk et al. 2020; Masondo and Makunga 2019; Rodrigues-Amaya 2019; Peschel and Politi 2015; Zhou et al. 2014).

Depending on the origin of the *A. brasiliana*, it is possible for it to present different phytochemical compositions in the leaf extract and different antioxidant and antimicrobial activities (Coutinho et al. 2018; Deladino et al. 2017; Trapp et al. 2015). However, reports about antibacterial activity of *A. brasiliana* extracts against *Mycobacterium smegmatis* have been scarce. This bacterium is used as bacterial model to study the effect of herbal extracts against tuberculosis, disease of high incidence in developing countries (Tawde et al. 2012). The antimycobacterial activity of plant extracts belonging to the Amaranthaceae family has previously been published, such as *Salicornia europaea* and *Gomphrena celosioides* (Hrynkiewicz et al. 2019; Omokhua et al. 2018).

In addition to the studies about the pharmacological activity of medicinal plants used in traditional folk medicine, it is very important to evaluate the cytotoxicity of these herbal extracts for greater safety of use (Coutinho et al. 2018; Omokhua et al. 2018; Ullah et al. 2013).

Therefore, the objective of this study is to evaluate the presence and type of carbohydrates and phenolic molecules in the aqueous extract (Aq-E) of *Alternanthera brasiliana* fresh leaves and its antioxidant and antimicrobial activities and cytotoxicity in vitro.

Results and discussion

Chemical composition of the aqueous extract (Aq-E) from *A. brasiliana* leaves

Aq-E is rich in carbohydrates (203.89 ± 20.66 µg/mg dw), among them only 0.1% are reducing sugars (0.228 ± 0.006 µg/mg dw) that represent free mono and disaccharides, and 24.27% (49.597 ± 0.398 µg/mg dw) are uronic acids. The extract also contains high amount of phenolic compounds (43.85 ± 1.03 µg/mg dw) (Table 1).

However, the question is: what is the amount of these compounds in one leaf? since Brazilian communities use different numbers of fresh leaves to prepare the aqueous extract, tea or plasters. To answer the question, the mean weight of one fresh leaf of *A. brasiliana* (589.900 ± 0.043 mg) was obtained as well as the dry weight (11.86 ± 1.11 mg), resulting in 88.14 ± 1.11% of moisture. Based on the fresh leaf weight, there is one leaf per 2 mL of Aq-E, allowing the calculation that each leaf contains approximately 3.09 mg of sugars; 3.46 µg of reducing sugars; 0.75 mg of uronic acids and 0.66 mg of phenolic compounds (Table 1).

The carbohydrates detected above were qualitatively identified by TLC in which were visualized the specific spots of glucose, fructose, sucrose, sugar acids (Rf = 0 similar red

Table 1 Amount of biomolecules amount in the aqueous extract of *Alternanthera brasiliana* leaves (295 mg of fresh leaf/mL or 34.98 mg of leaf dry weight/mL)

Biomolecules	Aq-E (μg/mg dw)	Fresh leaves (μg/mg)	Dry leaves (μg/mg)	Amount in one fresh leaf (μg)
Total sugars	203.93 ± 20.66 GE	5.2399 ± 0.5309	44.1902 ± 4.4775	3091.02 ± 225.87
Reducing sugars	0.228 ± 0.006 GE	0.00587 ± 0.0001	0.0495 ± 0.0012	3.46 ± 0.25
Uronic acids	49.597 ± 0.398 GLUAE	1.2744 ± 0.0102	10.7475 ± 0.0865	751.77 ± 54.93
Total phenolic compounds	43.85 ± 1.03 GAE	1.1267 ± 0.0265	9.5018 ± 0.2232	664.64 ± 48.57

Mean ± SD, n = 3

GAE gallic acid equivalent, *GE* glucose equivalent, *GLUAE* glucuronic acid equivalent, *BSAE* bovine serum albumin equivalent

spot pectin standard) and sugar alcohol (Rf = solvent line) compared to standards (Fig. 1a–c).

There is no previous reports about the soluble carbohydrates in the leaf extracts of *A. brasiliana*, meaning the current results are a scientific novelty.

However, a previous review by Kumar et al. (2011) reported the nutritional centesimal composition of the edible *A. brasiliana* leaf, showing moisture of 77.4% and carbohydrate amount of 11.6% of the leaf, not extract of leaf. The moisture of the leaves in the present work was higher than that reported by these authors, but the carbohydrate amount is not comparable because the total soluble carbohydrates in aqueous extract was measured free of the cellulosic residue. Another study reported the amount of carbohydrate in the nutritional composition of the wild Amaranthacea halophytes which belong to other genus of this family and has been consumed as a gourmet vegetables (Zaier et al. 2020).

The high amount of total sugars and uronic acids in the Aq-E can be related to the presence ß-O-glucose, glucuronic acid and ß-O-glucuronic acid in acylated betacyanin types, such as betanidin 5-O-(2'-O-(glucuronosyl) glucoside or betanidin 6-O-(-glucoside) acylated with ferulic, p-coumaric or 3-hydroxy-3-methylglutaric acid (Deladino et al. 2017; Khan and Giridhar 2015; Cai et al. 2005), in which the glycosidic bonds were hydrolysed by sulphuric acid used as reagent. The very low amount of reducing sugar, which is related to free mono and oligosaccharides in the Aq-E, can be explained by the fact that most mono and oligosaccharides are linked to betalains and flavonoids. Studies about carbohydrate composition of the aqueous or hydroalcoholic extracts of *A. brasiliana* leaves are scarce, possibly because this plant attracts attention for its green-purple leaves rich in betalain pigments and flavonoids are found in plant extracts as flavonoid glycosides (Rodriguez-Amaya 2019; Coutinho et al. 2018; Deladino et al. 2017). Although the sugar moieties are very important to intestinal absorption of these phenolic compounds, they are quickly hydrolysed by bacterial enzymes in the intestinal tract, producing flavonoid aglycones, non-glycoside betalains and free sugars and uronic acids, before their absorption through intestinal mucosa to physiological circulation for hepatic metabolism (Morris and Zhang 2006). Several reports show that carbohydrates, mainly uronic acids, play an important role as antioxidant,

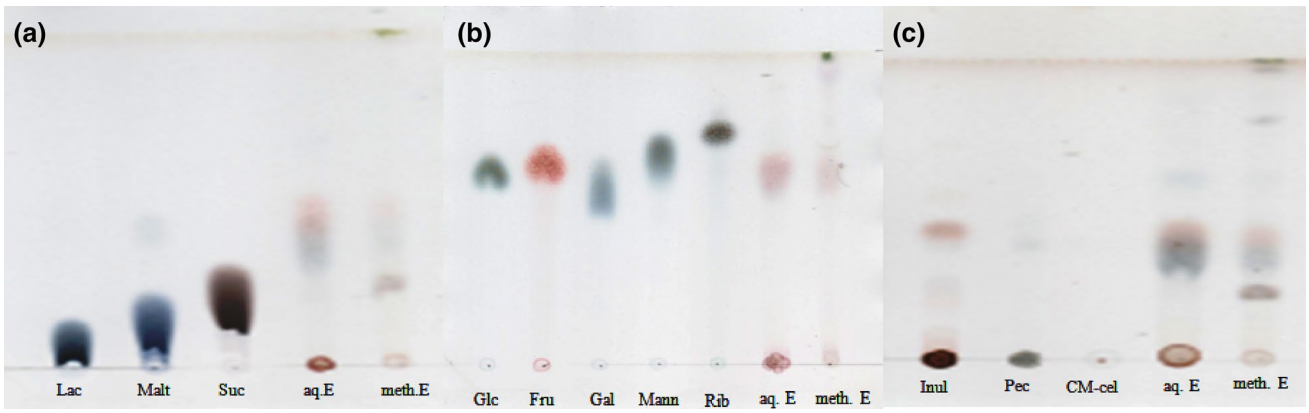

Fig. 1 **a–c** Thin Layer Chromatography (TLC) profile of aqueous (Aq-E) and methanol (met.E) extracts from *A. brasiliana* leaves. The standards used were: **a** glucose (Glu); fructose (Fru); galactose (Gal); mannose (Man); ribose (Rib). **b** Lactose (Lac); maltose (Malt); sucrose (Suc). **c** inulin (Inul); pectin (Pec); cellulose (CM-Cel)

antimicrobial and healing agent (Bayar et al. 2016; Kanchana et al. 2013; Huang et al. 2012; Aoyagi et al. 2008). Thus, it is possible that their association with phenolic compounds could amplify medicinal effect of *A. brasiliana* extracts, justifying the use by Brazilian communities as folk medicine.

Despite the total amount of phenolic compounds, the Aq-E showed higher values than the concentrations reported by Pereira et al. (2013) for ethanolic crude extract (32.7 ± 0.36 mg/g) and the value was twice as high as the concentration found by Deladino et al. (2017) using acidic acetonitrile:water extract (18.15 ± 0.59 mg GAE/g). However, the value was lower than the total phenolic concentration of the water extract of *A. Sesillis* (red) (58.99 ± 1.95 mg GAE/g) reported by Hazli et al. (2019).

HPLC profile of the Aq-E from A. brasiliana fresh leaves

The HPLC profile at 536 nm (Fig. 2a) of the Aq-E presented specific peaks of the Amaranthaceae chemical markers amaranthin (RT = 11.535 min), isoamaranthin (RT = 12.469 min), betanin (RT = 13.544 min) and iresinin I (RT = 17.581 min). In the HPLC profiles at 256 nm (Fig. 2b, c), several peaks of phenolic compounds were observed (Fig. 2b), where the highest peak at RT = 9.786 min; average peaks at RT = 4.225 min, RT = 8.945 min, RT = 9.588 min, RT = 11.776 min, RT = 12.358 min and RT = 13.766 min; and lowest peaks were detected at RT = 10.962 min and RT = 11.531 min. The highest peak of flavonoid (Fig. 2c) was obtained

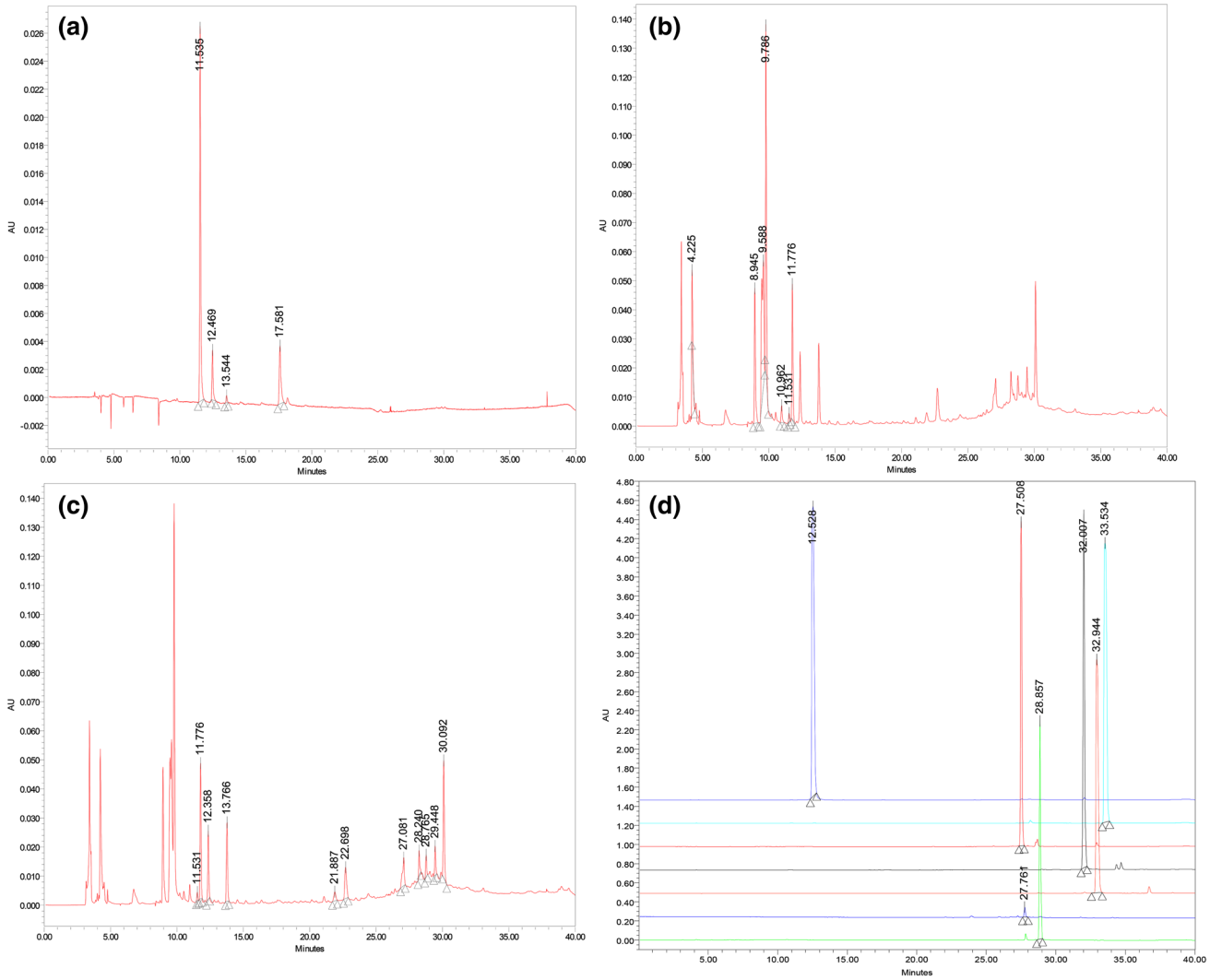

Fig. 2 **a–d** HPLC profiles of Aq-E from *A. brasiliana* leaves at 536 nm (**a**) and 256 nm (**b**, **c**). The overlay of phenolic compound standards (**d**) using gallic acid (RT = 12.528 min) and tannic acid (RT = 27.761 min) as acid phenolic standards and kaempferol (RT = 28.857 min), O-methyl-quercetin (RT = 32.944 min), quercetin (RT = 32.007 min), rutin (RT = 27.508 min), and cinnamic acid (RT = 33.534 min)

at RT = 30.092 min; average peaks at RT = 22.698 min, RT = 27.081 min, RT = 28.240 min, RT = 28.765 min, RT = 29.448 min; and the lowest peak at RT = 21.887 min. The HPLC profiles at 256 nm of the Aq-E were compared to the profile of the standards of phenolic acids and flavonoids in the same assay condition (Fig. 2d). The RT values of the peaks of the Aq-E obtained at 536 nm were compared to those reported by Cai et al. (2005) to identify the respective chemical markers of the Amaranthaceae family. These results are also similar to those showed by Khan and Giridhar (2015) and Deladino et al. (2017).

In addition, the results of the HPLC analysis can be applied to indicate the geographical origin and quality control of commercial herbal teas because the difference of the chemical characteristics due to soil composition, climate, sunlight, endophytic microorganisms and aqueous or organic solvent extract (Hrynkiewicz et al. 2019; Trapp et al. 2015, Macedo et al. 2014). During the development of this study, it was possible to obtain different HPLC profiles of the Aq-E obtained from *A. brasiliana* fresh leaves cultivated in different regions of Brazil, due to their different of phytochemical compositions. The sample cultivated in the South (Porto Alegre – RS) showed the highest amount of the amaranthin compared to the Northeast (Recife – PE) and Southeast (São Paulo – SP) samples and the sample from the Southeast did not show iresinin, while the Northeast sample presented the highest peak of this chemical marker (data not shown). Recently, the effect of cultivation conditions on phytocompound production by the plants was also demonstrated in a report about the LC-MS/MS and GC-MS analysis of the ethanolic extract of *A. brasiliana* leaves cultivated in the Northeast semi-arid region of Pernambuco (PE) State. The results showed five flavonoids isolated by LC-MS/MS, of which one has an unpublished structure, and twenty two compounds isolated from GC-MS which belong to several classes of chemical substances (hydrocarbons; mono, di and triterpenes; vitamin and carotenoid derivatives and phytosterols), which are also unpublished according to Alencar-Filho et al. (2020). These experimental data confirm that according to geographical origin and cultivation conditions, *A. brasiliana* can contain different phytocompounds giving its extracts several biological activities depending on the chemical and physical-chemical properties and association/synergism between these substances.

In addition, HPLC profiles have been applied to confirm the origin and quality of commercial honey and herbal products based on the presence and amount of the major compound groups characteristic of flowers or plant families (Shevchuk et al. 2020; Masondo and Makunga 2019; Peschel and Politi 2015; Zhou et al. 2014).

Antioxidant activity of the Aq-E from *A. brasiliana* fresh leaves

The result of Aq-E, expressed equivalent to TEAC, showed significant antioxidant activity of with the 51.31 ± 0.29% (1054.66 ± 58.92 µM Trolox) after 6 min, using 30 µL of Aq-E or 4651.051 ± 259.837 mM Trolox/g Aq-E (considering 0.227 mg of lyophilized Aq-E) (Table 2). The inhibition percentage was increased to 94.57 ± 0.29% after 120 min of reaction.

The antioxidant activity of the Aq-E using ABTS radical was excellent comparing to the values obtained by Hazli et al. (2019) for aqueous extract of *A. sesillis* leaves (0.28 ± 0.04 mmol TE/g) after 6 min of incubation with 3 µL extract (1 mg/mL). In addition, Pereira et al. (2013) showed low and dose-dependent antioxidant activity using DPPH radical of the ethanolic and organic solvent fractions from *A. brasiliana* leaves.

The total antioxidant activity by complexation of phosphomolybdenum was 38.60 ± 0.22% TAC after 30 min, utilizing lyophilized Aq-E solution (1 mg/mL), compared to acid ascorbic (1 mg/mL) as standard.

The different between the results of antioxidant activities by phosphomolybdenum complexation and ABTS free radical assays may be due to the transference mechanism of electrons/hydrogen from antioxidant compounds to each oxidant molecule, which varies according to the chemical structure and association/synergism between the Aq-E phytomolecules. According to Enechi et al. (2013), the total antioxidant activity based on metal complexation or chelation may be due to the antioxidant power of the total phenolic compounds. In addition, flavonoids are be able to produce complexes with heavy metal ions (Morris and Zhang 2006). However, it has been showed that other non-phenolics and non-flavonoid biomolecules can be used as a potent antioxidant agents, such as high concentration of uronic acid obtained by hydrolysis of the hyaluronic acid from bivalve mollusks (*Amussium pleuronectus*) which showed antioxidant activities towards ABTS (71.35% after 2 h), DPPH

Table 2 Antioxidant activity ($ABTS^+$) of Aq-E of *Alternanthera brasiliana* leaves

Time	% Inhibition	TEAC (µM Trolox)[a]
6 min	51.31 ± 2.47	1054.66 ± 58.92
15 min	59.35 ± 0.60	1329.66 ± 103.71
30 min	76.86 ± 3.76	1662.99 ± 89.57
45 min	74.20 ± 4.75	1599.66 ± 113.13
60 min	77.84 ± 5.14	1686.33 ± 122.57
120 min	94.57 ± 0.29	2084.66 ± 7.07

Mean ± SD, n = 3

[a]*TEAC* antioxidant activity equivalent to Trolox

(54.42%) and hydroxyl radicals (63.42%) compared to ascorbic acid as standard (Kanchana et al. 2013). In addition, the isolated polysaccharides with the highest content of uronic acid showed stronger antioxidant activities against superoxide anion, hydroxyl radical, and Ferric-reducing antioxidant power (FRAP) in a concentration-dependent manner, obtaining the highest activity using FRAP systems for the richest fraction of uronic acid (Huang et al. 2012).

Thus, it is possible to conclude that the high antioxidant activity of the Aq-E may be due to the association/synergism of the high concentration of phenolic and non-phenolic compounds.

Antimicrobial activity of Aq-E from *A. brasiliana* fresh leaves

The antimicrobial activity (in vitro) of lyophilized Aq-E against 12 microbial strains was qualitatively and quantitatively expressed by the MIC, and MBC values. The results were compared to standard antibiotics (Amoxicillin, erythromycin, ciprofloxacin and ketoconazole) used as positive controls (Table 3).

The most interesting and best results were obtained against *M. smegmatis* (71-UFPEDA) which was sensitive to Aq-E with an MIC of 15.6 µg/mL and MBC of 1000 µg/mL and *C. albicans* (1007-UFPEDA) with an MIC of 31.2 µg/mL. The Aq-E also showed positive results against other microorganisms assayed, with MIC values for: *S. aureus* (ATCC 6538) (2000 µg/mL), *M. luteus* (ATTC 2225) (2000 µg/mL), *P. aeruginosa* (39-UFPEDA) (1000 µg/mL), *E. faecalis* (ATCC 6057) (2000 µg/mL) and *P. aeruginosa* (CI, 736-UFPEDA, wound Secretion-HC) (2000 µg/mL). However, the Aq-E did not demonstrate antimicrobial activity for *B. subtilis* (16-UFPEDA), *E. coli* (ATCC 25,922), S*erratia sp*, (398-UFPEDA) *S. aureus* (CI, 731-UFPEDA, wound Secretion-HC, ORSA), *E. aerogenes* (CI, 739-UFPEDA, wound Secretion-HC).

According to Silva et al. (2011), there is a discussion about the use of the antibiotic standard (isolated substance) to compare the antimicrobial activity of vegetal extracts (a complex mixture of phytomolecules), while some researchers believe that comparing this activity with commercial antibiotics could make it possible to evaluate their antibiotic potential increasing their values as future herbal medicine. In general, when the vegetal extract shows action at concentrations above 1000 mg/mL it has weak antibiotic activity, while activity at concentrations lower than 100 mg/mL, can be used for further antimicrobial studies and evaluation of its antibiotic potential.

The Aq-E of *A. brasiliana* showed excellent antibiotic potential against *M. smegmatis* (71-UFPEDA) and antimycotic activity against *C. albicans* (1007-UFPEDA), with results better than those obtained by Silva et al. (2011) which showed MIC = 1.56 mg/mL for *C. albicans*, using the ethyl acetate fraction of *A. brasiliana*.

According to Hrynkiewicz et al. (2019), the great variety of endophytic microbiota increased the tolerance of the halophyte Amaranthaceae (*S. europaea*) to grow in soil with high salinity due to morphological and biochemical adaption, producing several bioactive metabolites, and Mycobacterium was one of the 37 genera found in this plant. In addition, the ethanolic and boiling water extracts of the other plant belonging to the Amaranthaceae family (*G. celosioides*) which is considered an alien invasive plant in South Africa, showed antimycobacterial activities (0.625 mg/mL and 1.25 mg/mL, respectively) (Omokua et al. 2018).

Table 3 The Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of Aq-E of *Alternanthera brasiliana* leaves and reference drugs tested for twelve microorganisms

Microorganisms	*A. brasiliana* (MIC/MBC) (µg/mL)	Drugs (MIC/MBC) (µg/mL)
Staphylococcus aureus (ATCC6538)	2000/ND	3.9/124 (*amoxicillin*)
Micrococcus luteus (ATTC 2225)	2000/ND	3.9/500 (*erythromycin*)
Bacillus subtilis (16-UFPEDA)	ND/ND	3.9/2000 (*erythromycin*)
Pseudomonas aeruginosa (39-UFPEDA)	1000/ND	3.9/31.25 (*ciprofloxacin*)
Mycobacterium smegmatis (71-UFPEDA)	15.6/1000	3.9/250 (*Amoxicillin*)
Enterococcus faecalis (ATCC6057)	2000/ND	3.9/250 (*Amoxicillin*)
Escherichia coli (ATCC25922)	ND/ND	3.9/7,81 (*ciprofloxacin*)
Serratia sp (398-UFPEDA)	ND/ND	3.9/250 (*ciprofloxacin*)
Candida albicans (1007-UFPEDA)	31.2/ND	3.9/62.5 (*ketoconazole*)
Staphylococcus aureus (CI, 731-UFPEDA, wound Secretion-HC, ORSA)	ND/ND	7.81/2000 (*amoxicillin*)
Pseudomonas aeruginosa (CI, 736-UFPEDA, wound Secretion-HC)	2000/ND	15.62/2000 (*ciprofloxacin*)
Enterobacter aerogenes (CI, 739-UFPEDA, wound Secretion-HC)	ND/ND	7.81/125 (*ciprofloxacin*)

CI clinical isolates, *HC* Public Clinical Hospital, *MIC* minimum inhibitory concentration (MIC, µg/ml), *MBC* minimum bactericidal concentration (MBC, µg/ml), *ND* not detect MIC or MBC

It has been reported that an antibiotic fraction extracted from *(A) brasiliana* stems, rich in linoleate oxylipins, was efficient against endophytic bacteria of the genus Bacillus isolated from the same plant. The antibiotic produced by these endophytic bacteria may be involved in an ecological relationship between this plant and its endophytic microorganisms. The isolated oxylipin fractions inhibited *(B) subitilis*, *M. luteus* and *S. aureus* at an MIC of 50 µg/mL (Trapp et al. 2015).

The antimycobacterial activity of the Aq-E is also better than those reported by Tawde et al. (2012) in five medicinal plants screened among the Indian plants used in traditional folk medicine for the treatment of tuberculosis and asthma, which inhibited *M. tuberculosis* growth at concentrations of 20 mg/mL and 40 mg/mL.

It is possible that the effects of soil composition, climate, and sunlight on the endophytic microbiota of the *A. brasiliana* from the Brazilian northeast region may explain our findings about the MIC (15.6 µg/mL) and (MBC = 1000 µg/mL) of Aq-E against *M. smegmatis* (71-UFPEDA) due to the production of antimycobacterium compounds from microbial biochemical metabolism.

The antibacterial activity of the ethanolic extract, rich in luteolin, against *E. coli*, *S. aureus* and *P. aeruginosa* was detected at concentration higher than 1024 µg/mL, which was considered clinically irrelevant by the authors, who obtained better results using extract mixed with gentamicin (Coutinho et al. 2018). The result of the Aq-E was similar to that against *P. aeruginosa* shown in this report.

Another study using an hydroalcoholic extracts of stem and leaves from *A. brasiliana*, collected in the Southeast of Paraná – South Brazil, showed that the extract prepared with ethanol 70% of *A. brasiliana* leaves was capable of inhibiting the growth of *E. coli* (MIC = 33.22 mg/L) and MIC = 66.25 mg/mL for *S. aureus* and *P. aeruginosa* (Araújo and Onofre 2011). The results obtained using Aq-E, MIC of 2 mg/mL for *S. aureus* (ATCC6538), 1 mg/mL for *P. aeruginosa* (39-UFPEDA) and 2 mg/mL for *P. aeruginosa* (CI, 736-UFPEDA, wound secretion-HC), were excellent compared to those obtained in the above cited report, and also better than the results reported by Pereira et al. (2013) that showed MIC values of ethanolic extract above 1250 µg/mL for all microorganisms tested, mainly against *C. albicans*.

Based on the previous reports cited here, the result obtained with Aq-E, a complex mixture of hydrophilic and hydrophobic phytomolecules, against *C. albicans* (MIC = 31.2 µg/mL), suggests further studies on its possible use as a potential anticandidal herbal medicine.

The results of the antimicrobial activities of the Aq-E of the *A. brasiliana* fresh leaves show the future possibility of this plant to be evaluated as a natural anti-inflammatory, alone or conjugated with allopathic antibiotics, in the treatment of infectious diseases.

Cytotoxicity of the Aq-E from *A. brasiliana* fresh leaves

The results of the cytotoxicity assay against *Artemia salina* (Fig. 3) show the low toxicity of the Aq-E with an LC_{50} of 500 µg/mL, which began to be significant from 250 µg/mL. It is relevant to note that at concentrations below 250 µg/mL the extract did not show significant toxicity. Low cytotoxicity can be considered an important characteristic to use a plant extract for medicinal purposes or for herbal medicine formulations.

A previous report (Ullah et al. 2013) showed a moderate cytotoxicity for methanolic extract of *A. sessilis* leaves (19.82 µg/mL) using the same method, which was higher than that found for the Aq-E. The ethanolic extract of *A. brasiliana* showed cytotoxicity against *D. melanogaster* in the three multiple concentrations (10, 20 and 40 mg/mL) after 24 h (Coutinho et al. 2018), which was much higher than that found for Aq-E, although detected by different method. According to Omokhua et al. (2018) the ethanolic extract of *G. celosioides* (invasive Amaranthaceae) against *Artemia salina* showed some cytotoxicity in the range from 0.75 to 0.95 mg/mL, which was lower compared to results of the Aq-E (LC_{50} of 0.50 mg/mL).

Conclusion

The present work showed that the molecular association between the great diversity of compounds of the Aq-E of *A. brasiliana* fresh leaves gave the extract optimum antioxidant activity and excellent antimicrobial activity against *M. smegmatis* and *C.* albicans, with low cytotoxicity. Further possibilities for this plant extract will be to improve the pharmacological potential for developing new herbal

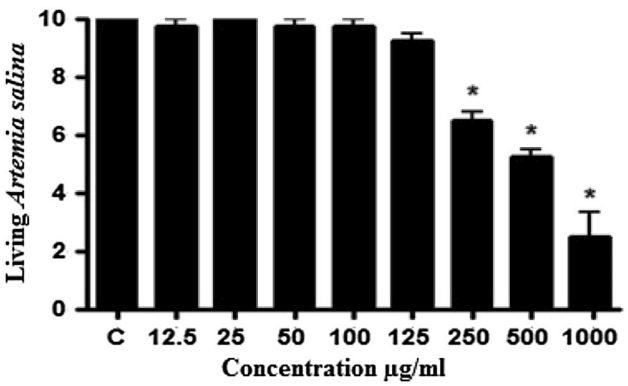

Fig. 3 Lethal cytotoxicity assay (LC_{50}) of Aq-E of *A. brasiliana* against *Artemia salina*. (*) Results that showed statistically significant differences compared to the control

medicines and possibly to study its association to allopathic antibiotics for prevention or treatment of infection diseases.

Experimental section

Plant material

The plant was identified by Dr Marlene Barbosa de Alencar (Botanic Department), being catalogued in the herbarium of the Federal University of Pernambuco (UFPE) under no. 21,846.

The sample of *A. brasiliana* fresh leaves was collected at greenhouse of the university in the city of Recife- PE, Brazil, which was processed at Laboratory of Immunopathology Keizo Asami (LIKA)-UFPE. All the chemical reagents were analytical grade.

Preparation of the extracts of *A. brasiliana* leaves

The aqueous extract (Aq-E) was prepared with 295 g fresh leaves (dry weight of 23.54 g) blended with 1 L of double-distilled water. The mixture was filtered, centrifuged for 30 min at 11,180 xg and the supernatant (Aq-E) was lyophilized (1.0 mL = 7.58 mg freeze-dried powder). The yield extraction was 32.20% (w/w). The methanol extract (Me-E) was prepared using 25 g of fresh leaves, which were cut in small pieces and placed in an Erlenmeyer flask containing 75 mL of methanol (1 g of fresh leaf : 3 mL of methanol) at 100 °C under stirring for 20 minutes. The Me-E was filtered and dried at 50 °C using a rotary evaporator. The residue was dissolved in methanol for further TLC assay.

Chemical composition of the Aq-E

The amount of total sugars was determined using phenol-sulphuric method (Dubois et al. 1956) and glucose as standard. Reducing sugars were measured using the DNSA method (Miller 1959) and glucose as standard. The carbazole-sulphuric method (Bitter and Muir 1962) was used to measure the concentration of uronic acids and glucuronic acid as the standard.

The amount of total phenolic compounds was determined according to the Folin–Ciocalteau method (Bin-Li et al. 2008), which was expressed in gallic acid equivalent (GAE).

Thin layer chromatography (TLC)

The types of sugar were qualitatively analysed by thin layer chromatography (TLC) according to Han and Robyt (1998) and Silva et al. (2002). The standard solutions (10 mg/mL) were glucose, fructose, galactose, mannose, ribose, sucrose, maltose, lactose, starch, inulin, pectin and CM-cellulose.

HPLC analysis of the Aq-E of *A. brasiliana* fresh leaves

The liquid chromatography profiles were obtained as described by Aboy et al. (2012), using the following systems: HPLC—Waters Alliance 2695 or 2690 chromatograph using PDA detector (UV/VIS Waters 2487) and C18 reversed-phase column (Luna® Phenomenex, 0.5 µm, 250 mm x 4.6 mm,) with a security guard column packed with Lichrosorb RP_{18} (10×4 mm; Merck). Waters Empower software was used for equipment control, data acquisition, and integration of the spectra obtained at 25 ± 2 °C and recorded in a range of 230–600 nm. Samples of Aq-E were injected and submitted at different gradient elutions of mobile phase formed by: (A) 0.01% (v/v) trifluoracetic acid in ultrapure water; (B) 0.08% (v/v) trifluoracetic acid in acetonitrile and (C) 100% methanol (Table 4). All solutions were filtered through a 0.45 µm membrane (Millipore) before use. The optimum parameters to have the best profiles at 256 nm and 536 nm were obtained using the following solvents at the flow rate of 0.7 mL/min: (A) 0.01% (v/v) trifluoracetic acid in ultrapure water; (B) 0.08% (v/v) trifluoracetic acid in acetonitrile and (C) 100% methanol according to isocratic gradient: A 100%:B 0%: C 0% (5 min); A 75%:B 15%: C 10% (10 min); A 50%:B 25%: C 25% (10 min); A 25%:B 25%: C 50% (10 min); A 50%:B 0%: C 50% (5 min).

Table 4 HPLC experimental conditions used for analysing the aqueous extract control of *Alternanthera brasiliana*. Mobile phases: (A) 0.01% (v/v) trifluoracetic acid in ultrapure water; (B) 0.08% (v/v) trifluoroacetic acid in acetonitrile and (C) 100% methanol. HPLC profiles were obtained at 25 ± 2 °C and recorded in a range of 210–600 nm

Mobile phase composition	Sample (µL)	Flow rate (mL/min)
1. 100%A:0%B(10 min); 75%A:25%B(15 min); 50%A:50%B (15 min); 25%A:75%B(10 min)	10 (50x diluted)	0.5
2. 100%A:0%B(10 min); 75%A:25%B(15 min); 50%A:50%B (15 min)	10 (10x diluted)	0.7
3. 100%A:75%B (25 min); 50%A:50%B(15 min)	10	0.5
4. 100%A:75%B (25 min); 50%A:50%B(15 min)	20	0.7
5. 100%A:0%B:0%C (5 min); 75%A:15%B:10%C(10 min); 50%A:25%B:25%C(10 min); 25%A:25%B:50%C(10 min); 50%A:0%B:50%C(5 min)	20	0.7

Antioxidant activities of the Aq-E of *A. brasiliana* fresh leaves

ABTS.$^+$ assay

According to Re et al. (1999), the 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assay is based on the generation of chromophore cationic radical obtained from the oxidation of ABTS by potassium persulfate. Aliquots of Aq-E (30 µL = 0.2274 mg of lyophilized Aq-E) were mixed with 3 mL of diluted ABTS.$^+$ solution. The absorbance at 734 nm of each sample was read after defined time intervals (0, 6, 30, 60, 90, 150, and 180 min). Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) was used as standard. The values of oxidative inhibition percentage were calculated and plotted as a function of the reference antioxidant concentration (Trolox) and expressed as Trolox equivalent antioxidant capacity (TEAC, µM).

Phosphomolybdenum assay

The total antioxidant capacity (TAC) was evaluated by the phosphomolybdenum (*P–Mo*) method (Prieto et al. 1999). Aliquots of 100 µL of the Aq-E solution (1 mg lyophilized Aq-E/mL) were mixed with 1.0 mL of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The capped tubes were incubated at 90 °C for 90 min. The absorbances at 695 nm against a blank (1 mL of reagent and 0.1 mL of the solvent) were recorded. Total antioxidant activity (TAC) was expressed in relation to ascorbic acid (100 µL of 1 mg/mL solution) and calculated by the following equation:

$$\%TAC = (A_s - A_c)/(A_{aa} - A_c) \times 100$$

where A_c is the absorbance of the control (without extract), A_s is the absorbance of the extract and A_{aa} is the absorbance of ascorbic acid.

Microorganisms for antimicrobial activity

The antimicrobial activity was obtained against the following microorganisms: *Staphylococcus aureus* (ATCC6538), *Micrococcus luteus* (ATTC2225), *Bacillus subtilis* (16-UFPEDA), *Pseudomonas aeruginosa* (39-UFPEDA), *Mycobacterium smegmatis* (71-UFPEDA), *Enterococcus faecalis* (ATCC6057), *Escherichia coli* (ATCC25922), *Serratia sp* (398-UFPEDA), *Candida albicans* (1007-UFPEDA), *Staphylococcus aureus* (CI, 731-UFPEDA, wound Secretion-HC, ORSA), *Pseudomonas aeruginosa* (CI, 736-UFPEDA, wound Secretion-HC), and *Enterobacter aerogenes* (CI, 739-UFPEDA, wound Secretion-HC). The microorganisms were provided by the Department of Antibiotics of the Federal University of Pernambuco – UFPE – Brazil and stored at 4 °C until further use.

The microbial inoculum was standardized to 10^8 cells/mL, estimated by comparison to the scale tube 0.5 Mac Farland (0.05 mL of 1.0% barium chloride dihydrate to 9.95 mL in 1% sulphuric acid).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC is considered as the lowest concentration of the sample capable of inhibiting the visible microbial growth according to the Committee for Clinical Laboratory Standards Institute (CLSI 2002). The MIC was determined by the broth microdilution performed in 96-well ELISA plates. The aqueous extract was tested in different concentrations from 2000 up to 3.9 µg/mL obtained from the solution prepared with 18 mg of lyophilized Aq-E suspended in 900 µL of DMSO (2% w/v). Aliquots from each Aq-E concentration were mixed with the microbial broth contained in the well plate well and incubated under aerobic conditions at 37 °C for 24 h. The MIC values were evaluated after incubating the plates with 20 µL of resazurin solution (100 µg/mL) per well for 30 min. The MBC, although there is one yeast strain, was determined taking samples from the positive MIC, which was cultured on the new medium plates at 37 °C for 24 hours. Before and after the growth period, the turbidity of each ELISA plate was read using ELISA Plate Reader (BioTek, Mod. ELx808, Germany).

Samples of the powdered forms of Amoxicillin, erythromycin, ciprofloxacin and ketoconazole (Sigma - St. Louis, MO, USA) were used as reference antimicrobial drugs at the same concentrations as the extract (20 mg/mL).

Cytotoxicity

The cytotoxicity assay (LC_{50}) was performed according to Meyer et al. (1982), using *Artemia salina* and Aq-E samples in concentrations below 1000 µg/mL, which is capable of killing 50% of the larvae (LC_{50}).

Statistical analysis

The experiments were carried out randomly in triplicate or quadruplicate, presented as mean ± SD. The results were analysed using one-way ANOVA and the Newman–Keuls method with $p < .05$.

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Compliance with ethical standards

Ethical statement The authors declare that the present manuscript/data have not been published and are not currently under review for publication elsewhere.

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References

Aboy AL, Apel MA, Debenedetti S, Francescato L, Rosella AA, Henriques AT (2012) Assay of caffeoylquinic acids in *Baccharis trimera* by reversed-phase-liquid chromatography. J Chromatogr A 1219:147–153

Alencar-Filho JMT, Teixeira HAP, Sampaio PA, Pereira ECV, Amariz IA, Rolim-Neto PJ, Rolim LA, Araújo ECC (2020) Phytochemical analysis in *Alternanthera brasiliana* by LC-MS/MS and GC-MS. Nat Prod Res 34:429–433

Aoyagi H, Ishii H, Ugwu CU, Tanaka H (2008) Effect of the heat-generated product from uronic acids on the physiological activities of microbial cells and its application. Bioresour Technol 99:4534–4538

Araújo D, Onofre SB (2011) Ação do extrato hidroalcoólico de *Alternanthera brasiliana* (L.) O. Kunt., (Amaranthaceae) sobre a atividade de antimicrobianos utilizados na terapêutica. SaBios Rev Saúde Biol 6:1–8

Barua CC, Talukdar A, Begum SA, Sarma DK, Fathak DC, Barua AG, Bora RS (2009) Wound healing activity of methanolic extract of leaves of *Alternanthera brasiliana* Kuntz using *in vivo* and *in vitro* model. Indian J Exp Biol 47:1001–1005

Bayar N, Kriaa M, Kammoun R (2016) Extraction and characterization of three polysaccharides extracted from *Opuntia ficus indica* cladodes. Int J Biol Macromol 92:441–450

Bin-Li H, Wong CC, Cheng KW, Chen F (2008) Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT Food Sci Technol 41:385–390

Bitter T, Muir M (1962) A modified uronic acid carbazole reaction. Anal Biochem 4:330–334

Cai Y-Z, Sun M, Corke H (2005) Characterization and application of betalain pigments from plants of the Amaranthaceae. Trends Food Sci Techn 16:370–376

CLSI document M27-A2 [ISBN 1-56238-469-4] (2002) (CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA), Approved Standard—Sixth Edition

Coutinho HDM, Oliveira-Tintino CDM, Tintino SR, Pereira RLS, Freitas TS, Silva MAP, Franco JL, Cunha FAB, Costa JGM, Menezes IRA, Boligon AA, Rocha JBT, Rocha MI, Santos JFS (2018) Toxicity against *Drosophila melanogaster* and antidematogenic and antimicrobial activities of *Alternathera brasiliana* (L.) Kuntze (Amaranthaceae). Environ Sci Pollut Res 25:10353–10361

Deladino L, Alvarez I, De Ancos B, Sánchez-Moreno C, Molina-García AD, Teixeira AS (2017) Betalains and phenolic compounds of leaves and stems of *Alternanthera brasiliana* and *Alternanthera tenella*. Food Res Int 97:240–249

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Enechi OC, Odo CE, Wuave CP (2013) Evaluation of the in vitro antioxidant activity of *Alternanthera brasiliana* leaves. J Pharm Res 6:919–924

Formagio EL, Mendel MT, Fracasso R, Knobloch JG, Teixeira PW, Kehl L, Maluf RW, Picoli SU, Ardenghi P, Suyenaga ES (2012) Evaluation of the pharmacological activity of the *Alternanthera brasiliana* aqueous extract. Pharm Biol 50:1442–1447

Han NS, Robyt JF (1998) Separation and detection of sugars and alditols on thin layer chromatograms. Carbohydr Res 313:135–137

Hazli UHAM, Abdul-Aziz A, Mat-Junit S, Chee CF, Kong KW (2019) Solid-liquid extraction of bioactive compounds with antioxidant potential from *Alternanthera sesillis* (red) and identification of the polyphenols using UHPLC-QqQ-MS/MS. Food Res Int 115:241–250

Hrynkiewicz K, Patz S, Ruppel S (2019) *Salicornia europaea* L. as an underutilized saline-tolerant plant inhabited by endophytic diazotrophs. J Adv Res 19:49–56

Huang S-Q, Ding S, Fan L (2012) Antioxidant activities of five polysaccharides from *Inonotus obliquus*. Int J Biol Macromol 50:1183–1187

Kanchana S, Arumugam M, Giji S (2013) Isolation, characterization and antioxidant activity of hyaluronic acid from marine bivalve mollusk *Amussium pleuronectus* (Linnaeus, 1758). Bioact Carbohydr Diet Fibre 2:1–7

Khan MI, Giridhar P (2015) Plant betalains: chemistry and biochemistry. Phytochemistry 117:267–295

Kumar S, Singh P, Mishra G, Srivastav S, Jha KK, Khosa RL (2011) Phytopharmacological review of *Alternanthera brasiliana* (Amaranthaceae). Asian J Plant Sci Res 1:41–47

Macedo AF, Leal-Costa MV, Tavares ES, Lage CLS, Esquibel MA (2014) The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze. Environ Exp Bot 70:43–50

Masondo NA, Makunga NP (2019) Advancement of analytical techniques in some South African commercialized medicinal plants: Current and future perspectives. S Afr J Bot 126:40–57

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLauglin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Plant Medic 45:31–34

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Morris ME, Zhang S (2006) Flavonoid-drug interactions: effects of flavonoids on ABC transporter. Life Sci J 78:2116–2130

Omokhua AG, Madikizela B, Aro A, Uyi OO, Van Staden J, McGaw LJ (2018) Noxious to ecosystems, but relevant to pharmacology: four South African alien invasive plants with pharmacological potential. S Afr J Bot 117:41–49

Pereira DF, Zanon RB, Dos Santos M, Boligon AA, Athayde ML (2013) Antioxidant activities and triterpenoids isolated from *Alternanthera brasiliana* (L.) Kuntze leaves. Nat Prod Res 27:1660–1663

Peschel W, Politi M (2015) ^{1}H NMR and HPLC/DAD for *Cannabis sativa* L. chemotype distinction, extract profiling and specification. Talanta 140:150–165

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26:1231–1237

Rodríguez-Amaya DB (2019) Update on natural food pigment: a mini-review on carotenoids, anthocyanins, and betalains. Food Res Int 124:200–205

Shevchuk A, Megías-Pérez R, Zemedie Y, Kuhnert N (2020) Evaluation of carbohydrates and quality parameters in six types of commercial teas by targeted statistical analysis. Food Res Int 133:109122

Silva LC, Pegoraro KA, Pereira AV, Esmerino LA, Cass QB, Barison A, Beltrame FL (2011) Antimicrobial activity of *Alternanthera brasiliana* Kuntze (Amaranthaceae): a biomonitored study. Lat Am J Pharm 30:147–153

Silva NH, Maia MBS, Silva EF, Pereira EC, Catanho MTJ, Schuler AP (2002) Antinociceptive activity of crude extracts and atranorin obtained from the *Cladina dendroides* (des Abb.) Ahti. Acta Farm Bonaerense Argentina 21(4):259–264

Tawde KV, Gacche RN, Pund MM (2012) Evaluation of selected Indian traditional folk medicinal plants against *Mycobacterium tuberculosis* with antioxidant and cytotoxicity study. Asian Pac J Trop Dis 2:S685–S691

Trapp MA, Kai M, Mithöfer A, Rodrigues-Filho E (2015) Antibiotic oxylipins from *Alternanthera brasiliana* and its endophytic bacteria. Phytochemistry 110:72–82

Ullah MO, Haque M, Urmi KF, Md. Zulfiker AH, Anita ES, Begum M, Hamid K (2013) Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. Asian Pac J Trop Biomed 3:1–7

Zaier MM, Ciudad-Mulero M, Cámara M, Pereira C, Ferreira ICFR, Achour L, Kacem A, Morales P (2020) Revalorization of Tunisian wild Amaranthaceae halophytes: nutritional composition variation at two different phenotypes stages. J Food Compos Anal 89:103463

Zhou J, Yao L, Li Y, Chen L, Wu L, Zhao J (2014) Floral classification of honey using liquid chromatography-diode array detection-tandem mass spectrometry and chemometric analysis. Food Chem 145:941–949