



## Nutritional and phytochemical profiles and biological activities of *Moringa oleifera* Lam. edible parts from Guinea-Bissau (West Africa)

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### ABSTRACT

*Moringa oleifera* is an edible medicinal plant used to fight malnutrition in Africa. In this study, *M. oleifera* flowers, fruits and seeds from Guinea-Bissau were characterized for their nutritional composition and hydroethanolic and aqueous extracts were prepared to investigate the phenolic profiles and bioactivities. Seeds presented higher levels of proteins (~31 g/100 g dw), fat (~26 g/100 g dw) and flavan-3-ol derivatives, while carbohydrates, proteins, citric acid, and glycosylated flavonoids were abundant in fruits and flowers, these last samples also being rich in  $\alpha$ -tocopherol (~18 mg/100 g dw). Some of the identified polyphenols had never been described in *M. oleifera*. In general, hydroethanolic extracts contained more polyphenols and were more active against lipid peroxidation, NO production, and tumour cells growth. Significant antimicrobial effects against the tested bacteria and fungi strains were displayed by both hydroethanolic and aqueous extracts. The *M. oleifera* potential to fight malnutrition and health issues was highlighted.

### 1. Introduction

The search for plants and plant-based products that can face the raising necessities of food and medicines in a context of climate changes and food scarcity is nowadays a major challenge in Africa where persist malnutrition problems (Muyonga, Nansereko, Steenkamp, Manley, & Okoth, 2016). In this context, *Moringa oleifera* Lam. (Moringaceae) appears as a species with nutritional, medicinal and agronomic value. This fast-growing, deciduous tree is native to the Indian subcontinent and Pakistan, and has become naturalized in the tropical and sub-tropical areas around the world, namely in many African countries due to its easy adaptability and tolerance to a wide range of environmental conditions regarding climate and soil (Daba, 2016).

*M. oleifera* is one of the most auspicious plants used as a suitable alternative for preventing and alleviating malnutrition challenges, especially hidden hunger health issues (Padayachee & Bajinath, 2020). It is considered to be a "Miracle tree" or "Tree of life" due to the substantial beneficial effects that it has on health, but also due to its potential use in water sanitation and environmental conservation (Daba,

2016). *M. oleifera* preparations have been reported in the scientific literature as having a wide range of pharmacological properties, including antimicrobial, hypotensive, hypoglycemic, immunomodulatory, and anti-inflammatory activities. In addition, all *M. oleifera* parts (including leaves, fruits, seeds, pods, and flowers) have been used in traditional foods and dishes for human consumption (Daba, 2016).

The leaves and seeds are eaten fresh, powdered or cooked and contain a varied profile of nutrients and health-promoting compounds, such as fatty acids, tocopherols,  $\beta$ -carotene, and phenolic compounds. The fruits are fibrous and traditionally used to treat digestive problems and prevent colon cancer. Flower extracts, in turn, are used in culinary preparations to enhance the taste and colour of dishes (Padayachee & Bajinath, 2020; Ziani et al., 2019). These *M. oleifera* organs are also known to be good sources of secondary metabolites, including terpenoids, flavonoids, tannins, anthocyanins, and proanthocyanidins (Ajibade, Arowolo, & Olayemi, 2013). These bioactive compounds contribute to the therapeutic and medicinal properties of *M. oleifera* and may justify its uses by the indigenous system of medicine in the

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treatment of common ailments and disorders, such as anaemia, asthma, diarrhea, skin infections, headaches, swelling, hysteria, cholera, scurvy, respiratory disorders, diabetes, cough, sore throat, and chest congestion (Padayachee & Baijnath, 2020). Therefore, this edible medicinal plant appears as a natural remedy easily accessible to populations in developing countries that need basic healthcare, especially in areas where Western medicine is inaccessible or expensive (Padayachee & Baijnath, 2020). Curiously, *M. oleifera* seed powder is used as a purifying agent in the treatment of water, being able to eliminate pathogenic bacteria up to 99%, whereas fresh leaves can be used to extract a juice used as a growth hormone (or soil fertilizer) able to increase crop yields by 25–35% (Daba, 2016).

In Guinea-Bissau (West Africa), the awareness of local populations about the medicinal and nutritional properties of *M. oleifera* has increased in the last years, where the trade of seeds and dried and crushed leaves is under development. Despite this, the exploitation of the different edible and medicinal parts of this plant in this country is far to reach their full potential (Bancesi, Bancesi, Baldé, & Catarino, 2020). Therefore, due to the multiple traditional uses and applications of *M. oleifera*, this study was performed to determine the detailed nutritional and chemical composition (proximate constituents, free sugars, organic acids, tocopherols, fatty acids, and phenolic compounds) of seed, flower and fruit samples collected in two distinct locations in Guinea-Bissau using official methods of food analysis and advanced chromatographic techniques. In addition, the antioxidant, anti-inflammatory, cytotoxic, and antimicrobial activities of hydroethanolic, infused and decocted extracts prepared with the three *M. oleifera* organs were assessed *in vitro* using different cellular assays and food-borne microorganisms. In this way, it is intended to demonstrate and validate the food and medicinal potential of *M. oleifera*, which can have a direct impact on the food security of local African populations and be useful for the development of new functional foods and nutraceuticals.

## 2. Material and methods

### 2.1. Sampling and samples preparation

*M. oleifera* seeds, flowers and immature fruits (Fig. 1) were collected



Fig. 1. Edible parts of *Moringa oleifera* characterized in this study: a) flowers; b) seeds; and c) Immature fruits.

in early May 2019 in two locations in Guinea-Bissau: Granja (11° 52'02"N; 15° 36'06"W), a state farm inside Bissau urban area, and in a homegarden in Ponta Romana, Quinhamel, located in the countryside (11°54'18"N; 15°49'45"W). The two collecting sites are about 30 km apart and the soil and climatic conditions in both sites are similar (ferralsols, rainfall c. 1500 mm per year). The main differences are the urban vs. rural environment and the fact that in Granja the harvested trees were isolated, with direct sunlight during most of the day and in Ponta Romana the samples were taken from trees of a living fence in a homegarden, with less direct sunlight. The plant samples were then lyophilized (FreeZone 4.5, Labconco, MO, USA) and reduced to a fine powder that was stored in well-sealed plastic bags at  $-20^{\circ}\text{C}$  in the dark until further analysis.

### 2.2. Nutritional value and energy assessment

The *M. oleifera* edible samples were analysed for moisture, protein, fat, and ash contents following the AOAC analytical procedures (AOAC International, 2016). Total carbohydrates were calculated by difference and the energetic value was calculated according to the Regulation (EC) No. 1169/2011 of The European Parliament and of the Council as follows: energy (kcal/100 g dried weight (dw)) =  $4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat})$ .

### 2.3. Chromatographic analysis of free sugars, organic acids, fatty acids, and tocopherols

Free sugars were analysed in a high-performance liquid chromatography (HPLC) system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a refractive index detector (Smartline System 1000), using the internal standard (melezitose, Sigma-Aldrich, St Louis, MO, USA) method previously described by Spréa et al. (2020). Data were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and the results were expressed as g per 100 g dw.

Organic acids were analysed by ultra-fast liquid chromatography (Shimadzu 20A series, Shimadzu Corporation, Kyoto, Japan) coupled to a diode-array detector operating in the conditions described by Spréa et al. (2020). The compounds were identified by comparing their retention time and UV-Vis spectra with those of standards (oxalic, malic, ascorbic, citric, and fumaric acids, Sigma-Aldrich, St. Louis MO, USA) and quantified based on calibration curves obtained by plotting the peak area recorded at 245 nm for ascorbic acid and at 215 nm for the remaining acids against concentration. Data were recorded and processed using LabSolutions Multi LC-Photodiode Array (PDA) software (Shimadzu Corporation, Kyoto, Japan) and the results were given as g per 100 g dw.

The fatty acids profile was determined by gas-liquid chromatography (DANI 1000, Switzerland) coupled to a flame ionization detector (FID) operating in the conditions previously described by Spréa et al. (2020). Data were recorded and processed using Clarity 4.0 software and the results were given as relative percentage of each fatty acid.

Tocopherols were determined using the internal standard (tocol, Matreya, Pleasant Gap, PA, USA) method and the HPLC system (Smartline System 1000, Knauer, Berlin, Germany) coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, as previously described by Spréa et al. (2020). Data were recorded and processed using Clarity 2.4 software and the results were given as mg per 100 g dw.

### 2.4. Preparation of *M. oleifera* hydroethanolic and aqueous extracts

The *M. oleifera* seed, flower and immature fruit samples were prepared in hydroethanolic, infused and decocted extracts to evaluate their composition in phenolic compounds and the *in vitro* bioactive

properties. These preparation/extraction methods were selected according to the traditional uses of the different parts of the plant (Dhakar et al., 2011; Ilyas, Arshad, Saeed, & Iqbal, 2015; Lim, 2014).

To prepare the hydroethanolic extracts, each sample (2 g) was mixed with ethanol/water solution (80:20, v/v; 30 mL) and stirred for 1 h at room temperature. After filtering the supernatant through Whatman filter paper No 4, the residue was re-extracted and the combined filtrates were concentrated under reduced pressure (rotary evaporator Büchi R-210, Switzerland) at 40 °C and the aqueous phase was subsequently lyophilized (Iyda, Fernandes, Ferreira et al., 2019).

For decoctions, each sample (2 g) was boiled with distilled water (100 mL) for 5 min in heating plate (VELP Scientific) and then filtrated through Whatman filter paper No 4. The obtained decoctions were frozen and lyophilized (Iyda, Fernandes, Ferreira et al., 2019).

Only seeds and flowers were used to prepare infusions. The samples (2 g) were infused with freshly boiled distilled water (100 mL), left aside for 5 min and subsequently filtered through Whatman filter paper No 4. The resulting extracts were frozen and lyophilized (Adouni et al., 2018).

## 2.5. HPLC-DAD-ESI/MS<sup>n</sup> analysis of phenolic compounds

Phenolic compounds were analysed in hydroethanolic, infused and decocted extracts, which were redissolved in ethanol/water (80:20, v/v) and water, respectively, to a final concentration of 10 mg/mL and filtered using 0.22 µm disposable filter disks. The analysis was performed in a HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, California, USA) coupled with a diode-array detector (DAD, using 280 and 370 nm as preferred wavelengths) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Separation was made in a Waters Spherisorb S3 ODS-2 C18 column (3 µm, 4.6 mm × 150 mm; Waters, Milford, MA, USA). The operating conditions were previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016), as well as the identification and quantification procedures. The results were given as mg per g of extract.

## 2.6. Evaluation of bioactive properties in vitro

### 2.6.1. Antioxidant activity

Two cell-based assays were performed to measure the *in vitro* antioxidant activity of the extracts (0.1563–5 mg/mL), following methodologies formerly described by Spréa et al. (2020) and Lockowandt et al. (2019). The extracts capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS) was assessed using porcine brain cell tissues as oxidizable substrates, and the results were expressed as half maximal effective extract concentration (EC<sub>50</sub>) values (mg/mL). The oxidative haemolysis inhibition assay (OxHLIA) was performed to assess the extracts capacity to protect sheep erythrocytes from the AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride)-induced oxidative haemolysis. Half maximal inhibitory concentration (IC<sub>50</sub>) values (µg/mL) were calculated for time intervals (Δt) of 60 and 120 min and translate the extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control.

### 2.6.2. Nitric oxide (NO)-production inhibition activity

The anti-inflammatory activity of the extracts (at concentrations up to 400 µg/mL) was assessed based on the nitric oxide (NO) production by a lipopolysaccharide (LPS)-stimulated murine macrophage (RAW 264.7) cell line. The NO production was quantified based on the nitrite concentration using the Griess Reagent System kit containing sulphanilamide, N-1-naphthylethylenediamine dihydrochloride and nitrite solutions, following a procedure previously described by Corrêa et al. (2015). Dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) was used

as a positive control, while no LPS was added in negative controls. The effect of the tested extracts in NO basal levels was also assessed by performing the assay in the absence of LPS. The results were expressed as IC<sub>50</sub> values (µg/mL), which correspond to the extract concentration providing 50% of NO production inhibition.

### 2.6.3. Cytotoxic activity

The extracts cytotoxicity was assessed by the sulforhodamine B (Sigma-Aldrich, St. Louis, MO, USA) assay against four human tumour cell lines (acquired from Leibniz-Institut DSMZ), namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma), following a protocol previously described by Spréa et al. (2020). Ellipticine (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. The same assay was also used to evaluate the hepatotoxicity of the extracts against a non-tumour cell line (PLP2, porcine liver primary cells) obtained as described by Spréa et al. (2020). The extract concentration (µg/mL) causing 50% cell growth inhibition (GI<sub>50</sub>) was calculated and used to express the results.

### 2.6.4. Antimicrobial activity

The extracts were redissolved in 5% dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL and further diluted. The microdilution method (Soković, Glamočlija, Marin, Brkić, & van Griensven, 2010) was performed to assess the antimicrobial activity against the Gram-negative bacteria *Escherichia coli* (ATCC 35210), *Salmonella* Typhimurium (ATCC 13311) and *Enterobacter cloacae* (ATCC 35030), and the Gram-positive *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate) and *Listeria monocytogenes* (NCTC 7973). The antifungal activity was assessed against *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), and *Penicillium aurantiogriseum* (food isolate) (Corrêa et al., 2015). The minimum extract concentrations that completely inhibited bacterial growth (MICs) were determined by a colorimetric microbial viability assay, and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were also calculated. Streptomycin, ampicillin, ketoconazole and bifonazole (Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls, and 5% DMSO was used as a negative control.

## 2.7. Statistical analysis

Three samples were used for each analysis and all the assays were carried out in triplicate. The results were presented as mean values and standard deviation. A Student's *t*-test was applied to assess significant difference among plant samples with a different geographic origin (Quinhamel and Bissau), with  $\alpha = 0.05$ . In the bioactive assays, a one-way analysis of variance (ANOVA) was applied, followed by Tukey's HSD test, with  $\alpha = 0.05$ , to assess significant differences between hydroethanolic, infused and decocted extracts. The analysis was carried out using SPSS v. 22.0 program SPSS Statistics software (IBM Corp., Armonk, NY, USA).

## 3. Results and discussion

### 3.1. Nutritional composition of *M. oleifera* edible parts

Since the plants composition is affected by different factors, such as the edaphoclimatic conditions of the different growing sites, agricultural practices, harvesting period, and genetic characteristics, among others (Iyda, Fernandes, Calhella et al., 2019), the studied samples of *M. oleifera* were collected at two distinct locations in Guinea-Bissau. Table 1 presents the proximal composition of the *M. oleifera* seeds, flowers, and fruits collected in Quinhamel and Bissau. Carbohydrates were found to be major constituents in all studied samples; the highest

**Table 1**

Nutritional value and composition in free sugars, organic acids, main fatty acids, and tocopherols of *M. oleifera* edible parts.

	Seeds			Flowers			Fruits		
	Quinhamel	Bissau	Student's t-test	Quinhamel	Bissau	Student's t-test	Quinhamel	Bissau	Student's t-test
			p-value			p-value			p-value
Moisture (%)	np	np	–	81.4 ± 0.5	81.4 ± 0.1	0.851	79.0 ± 0.4	76.8 ± 0.9	0.006
Fat (g/100 g)	26.0 ± 0.1	26.6 ± 0.1	0.001	5.27 ± 0.07	5.02 ± 0.05	0.002	4.3 ± 0.1	2.67 ± 0.06	< 0.001
Proteins (g/100 g)	30.0 ± 0.6	31.88 ± 0.08	0.002	21.3 ± 0.4	19.83 ± 0.01	0.001	19.79 ± 0.04	19.49 ± 0.06	0.476
Ash (g/100 g)	2.8 ± 0.1	2.67 ± 0.01	0.001	7.93 ± 0.09	7.95 ± 0.07	0.346	6.31 ± 0.06	5.93 ± 0.05	< 0.001
Carbohydrates (g/100 g)	41.2 ± 0.3	38.85 ± 0.03	< 0.001	65.5 ± 0.3	67.2 ± 0.1	0.001	79.6 ± 0.1	71.91 ± 0.04	< 0.001
Energy (kcal/100 g)	518.3 ± 0.4	522.2 ± 0.5	< 0.001	394.6 ± 0.5	393.2 ± 0.1	0.007	396.3 ± 0.5	389.7 ± 0.3	< 0.001
Fructose (g/100 g)	nd	nd	–	2.19 ± 0.02	1.51 ± 0.01	< 0.001	3.00 ± 0.04	2.86 ± 0.04	0.003
Glucose (g/100 g)	0.15 ± 0.05	0.16 ± 0.04	0.651	6.01 ± 0.07	3.30 ± 0.04	< 0.001	8.02 ± 0.04	10.03 ± 0.08	< 0.001
Sucrose (g/100 g)	1.17 ± 0.04	1.70 ± 0.03	< 0.001	2.93 ± 0.09	5.52 ± 0.07	< 0.001	5.03 ± 0.04	4.92 ± 0.01	0.005
Trehalose (g/100 g)	nd	nd	–	0.82 ± 0.03	0.75 ± 0.01	0.005	0.63 ± 0.01	1.01 ± 0.05	< 0.001
Total sugars (g/100 g)	1.32 ± 0.09	1.86 ± 0.06	< 0.001	12.0 ± 0.2	11.1 ± 0.1	0.001	16.7 ± 0.1	18.8 ± 0.2	< 0.001
Oxalic acid (g/100 g)	10.44 ± 0.05	10.6 ± 0.2	0.153	0.77 ± 0.01	1.82 ± 0.01	< 0.001	0.66 ± 0.01	1.18 ± 0.01	< 0.001
Malic acid (g/100 g)	nd	nd	–	1.79 ± 0.02	1.29 ± 0.02	< 0.001	1.84 ± 0.03	1.30 ± 0.01	< 0.001
Ascorbic acid (g/100 g)	nd	nd	–	0.25 ± 0.01	0.19 ± 0.01	< 0.001	0.35 ± 0.01	0.65 ± 0.02	< 0.001
Citric acid (g/100 g)	nd	nd	–	3.05 ± 0.01	3.12 ± 0.02	0.001	1.84 ± 0.02	2.62 ± 0.01	< 0.001
Fumaric acid (g/100 g)	tr	tr	–	tr	tr	–	tr	tr	–
Total organic acids (g/100 g)	10.44 ± 0.05	10.6 ± 0.2	0.153	5.85 ± 0.01	6.42 ± 0.01	< 0.001	4.71 ± 0.02	5.75 ± 0.02	< 0.001
C16:0	6.1 ± 0.2	7.0 ± 0.2	0.002	19.7 ± 0.1	21.6 ± 0.2	< 0.001	12.8 ± 0.2	10.4 ± 0.2	< 0.001
C18:0	5.53 ± 0.06	6.5 ± 0.2	< 0.001	4.64 ± 0.01	4.23 ± 0.09	< 0.001	4.67 ± 0.06	4.73 ± 0.07	0.221
C18:1n9	71.6 ± 0.2	69.4 ± 0.4	< 0.001	25.8 ± 0.1	20.32 ± 0.01	< 0.001	52.4 ± 0.6	48.8 ± 0.1	< 0.001
C18:2n6	0.65 ± 0.03	0.69 ± 0.06	0.192	15.1 ± 0.1	14.4 ± 0.5	0.023	7.42 ± 0.08	8.5 ± 0.3	< 0.001
C18:3n3	0.21 ± 0.02	0.195 ± 0.005	0.116	16.4 ± 0.1	22.3 ± 0.3	< 0.001	6.67 ± 0.09	6.3 ± 0.4	< 0.001
C22:0	7.0 ± 0.2	6.98 ± 0.09	0.446	5.6 ± 0.2	5.4 ± 0.5	0.414	7.43 ± 0.09	9.1 ± 0.1	< 0.001
C24:0	1.43 ± 0.08	1.33 ± 0.01	0.039	6.0 ± 0.3	5.0 ± 0.3	0.005	1.67 ± 0.09	3.6 ± 0.2	< 0.001
SFA (%)	24.1 ± 0.2	26.0 ± 0.4	0.001	41.0 ± 0.4	40.84 ± 0.08	0.366	31.3 ± 0.2	33.4 ± 0.5	< 0.001
MUFA (%)	75.1 ± 0.2	73.1 ± 0.5	0.001	26.6 ± 0.1	21.23 ± 0.04	< 0.001	55.0 ± 0.6	49.0 ± 0.1	< 0.001
PUFA (%)	0.86 ± 0.01	0.89 ± 0.06	0.299	32.4 ± 0.2	37.9 ± 0.1	< 0.001	14.4 ± 0.2	17.5 ± 0.6	< 0.001
α-Tocopherol (mg/100 g)	2.22 ± 0.02	3.36 ± 0.01	< 0.001	18.90 ± 0.01	17.22 ± 0.09	< 0.001	3.13 ± 0.05	4.67 ± 0.02	< 0.001
δ-Tocopherol (mg/100 g)	0.48 ± 0.01	1.48 ± 0.03	< 0.001	2.08 ± 0.01	2.68 ± 0.07	< 0.001	0.45 ± 0.04	0.19 ± 0.01	< 0.001
Total tocopherols (mg/100 g)	2.71 ± 0.01	4.84 ± 0.01	< 0.001	20.98 ± 0.01	19.90 ± 0.01	< 0.001	3.58 ± 0.09	4.86 ± 0.03	< 0.001

np - not performed; nd - not detected; tr - traces; C16:0 - palmitic acid; C18:0 - stearic acid; C18:1n9 - oleic acid; C18:2n6 - linoleic acid; C18:3n3 - α-linolenic acid; C22:0 - behenic acid; C24:0 - lignoceric acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

levels were detected in the fruit ( $71.91 \pm 0.04$  and  $79.6 \pm 0.1$  g/100 g dw) and the lowest in the seeds ( $38.85 \pm 0.03$  and  $41.2 \pm 0.3$  g/100 g dw in samples from Bissau and Quinhamel, respectively). Proteins rank second with the seeds showing the higher levels ( $30.0 \pm 0.6$  –  $31.88 \pm 0.08$  g/100 g dw), followed by the flower and the fruit. These last two plant parts also had an interesting content of ash (total minerals), which ranged from  $19.83 \pm 0.01$  to  $21.3 \pm 0.4$  g/100 g dw. As expected, the seeds had a higher fat content ( $\sim 26.3$  g/100 g dw) than the other two edible parts of *M. oleifera*. In addition, fruits collected in Quinhamel stood out with a significantly higher fat content ( $4.3 \pm 0.1$  g/100 g dw) than those collected in Bissau ( $2.67 \pm 0.06$  g/100 g dw). The results obtained in this study are slightly lower than those previously reported by Gopalakrishnan, Doriya, and Kumar (2016) and Liang, Wang, Li, Chu, and Sun (2019) for the fat (38.67 and 39.12 g/100 g) and protein (35.97 and 40.34 g/100 g) contents in Indian *M. oleifera* seeds, but were higher for carbohydrates (8.67 and 8.94 g/100 g).

Regarding the energy contribution, 100 g fruit and flower portions provide comparable values ( $\sim 390$ – $396$  kcal), while that of seeds were higher ( $\sim 518$ – $522$  kcal) mainly due to the fat content.

According to previous reports, *M. oleifera* oil can accelerate wound healing (Liang et al., 2019) and the seed protein fraction has potential to be used in surface water purification due to coagulant effects (Baptista et al., 2017). Therefore, *M. oleifera* edible parts arise as interesting possibilities for being exploited as raw materials for production of vegetable oil, protein-rich foods and skincare products.

As shown in Table 1, the chromatographic analysis allowed to

detect and quantify four free sugars in the studied *M. oleifera* flowers and fruits, namely fructose, glucose, sucrose and trehalose, while just glucose and fructose were found in the seeds. The highest levels were quantified in the fruits ( $16.7 \pm 0.1$  –  $18.8 \pm 0.2$  g/100 g fw), followed by the flowers ( $11.1 \pm 0.1$ – $12.0 \pm 0.2$  g/100 g fw) (Fig. S1, Supplementary material) and lastly by the seeds with significantly lower levels ( $1.32 \pm 0.09$  –  $1.86 \pm 0.06$  g/100 g fw). It was also noted that the quantitative sugar profile of the fruit and flower samples seemed to have been affected by their different origin. These differences could be attributed to edaphoclimatic factors and some biotic conditions that can affect biochemical and physiological processes involved in the plant sugars' production (Ziani et al., 2019). In a previous study, Ziani et al. (2019) identified fructose, glucose and sucrose in *M. oleifera* leaves from Algeria and reported a total free sugars content of 3.82 g/100 g dw. Upadhyay, Yadav, Mishra, Sharma, and Purohit (2015) described L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, and D-xylose as the predominant sugars in the purified whole-gum exudates of *M. oleifera*.

Regarding organic acids, the analysis allowed identifying oxalic, malic, ascorbic, citric, and fumaric acids in flower and fruit samples from both locations (Table 1). Citric and malic acids were the major compounds, while just traces of fumaric acid were detected. Fruits collected in Bissau contained a higher level of ascorbic acid ( $0.65 \pm 0.02$  g/100 g fw) than those from Quinhamel or the flower samples. The total organic acid contents ranged from  $4.71 \pm 0.02$  –  $5.75 \pm 0.02$  g/100 g fw in fruits to  $5.85 \pm 0.01$  –  $6.42 \pm 0.01$  g/100 g fw in flowers. In *M. oleifera* seeds,  $\sim 10.5$  g/100 g fw of oxalic acid



were quantified (Table 1), about twice the total content of organic acids found in the other two parts of the plant. Traces of fumaric acid were also detected. It is known that plant foods with a high oxalic acid concentration should be consumed moderately, because the high intake of oxalates may promote the formation of kidney stones, irritation of the intestinal mucosa, and also interferes with calcium absorption (Lyda, Fernandes, Ferreira et al., 2019). To the best of the authors' knowledge, no data are available in the literature regarding the organic acid composition of *M. oleifera* seeds, flowers or fruits. In leaves, Ziani et al. (2019) already reported oxalic, malic and ascorbic acids.

The main fatty acids identified in the studied *M. oleifera* edible parts are also presented in Table 1, while the detailed profiles are shown in Table S1 provided in Supplementary Material. Twenty-one fatty acids were identified in the fruit and flower lipid fractions, while just 14 were detected in the seed samples. The flower lipid fraction was mainly composed by unsaturated fatty acid (SFA; ~41%, due to the contribution of C16:0, C22:0 and C18:0), followed by polyunsaturated fatty acids (PUFA;  $32.4 \pm 0.2 - 37.9 \pm 0.1\%$ ), namely  $\alpha$ -linolenic (C18:3n3) and linoleic (C18:2n6) acids. *M. oleifera* fruits were abundant in monounsaturated fatty acids (MUFA;  $49.0 \pm 0.1 - 55.0 \pm 0.6\%$ ), particularly those collected in Quinhame homegardens, due to the high contents of oleic acid (C18:1n9), followed by SFA ( $31.3 \pm 0.2 - 33.4 \pm 0.5\%$ ), which predominated in the fruit samples from Bissau, given the high levels of palmitic (C16:0), behenic (C22:0) and stearic (C18:0) acids. MUFA also predominated in the seed samples ( $73.1 \pm 0.5 - 75.1 \pm 0.2\%$ ), mostly C18:1n9 but also minor levels of eicosenoic (C20:1) and palmitoleic (C16:1) acids. The SFA C16:0 and C22:0 were also detected in this plant part. In a previous work, Zheng, Wu, Peng, and Zhang (2019) studied the effects of soil drenching and foliar spraying of boron on *M. oleifera* seed oil quality and reported C18:1 levels ranging from 64.24 to 71.17%, a result comparable to that obtained in the present study ( $69.44 \pm 0.4$  and  $71.6 \pm 0.2\%$  for seeds from Bissau and Quinhame, respectively). The lipid composition of *M. oleifera* seeds is greater than that of soybean, which makes it nutritionally important and the refined seed oil is acceptable to substitute the olive oil because of the presence of all the essential fatty acids in it (Singh et al., 2020).

The tocopherols composition of the studied *M. oleifera* edible parts is shown in Table 1, where it can be seen that  $\alpha$ -tocopherol was the prevalent isoform in all samples, followed by  $\delta$ -tocopherol. The flower samples showed the highest  $\alpha$ -tocopherol concentrations, ranging from  $17.22 \pm 0.09$  to  $18.90 \pm 0.01$  mg/100 g dw (HPLC profile in Fig. S2, Supplementary material). Fruit and seed samples revealed a total content of tocopherols ranging from  $2.71 \pm 0.01$  to  $4.86 \pm 0.03$  g/100 g dw and the samples collected in Bissau showed higher levels of these lipophilic antioxidants. Singh et al. (2020) reported that tocopherols together with ascorbic acid, carotenoids and flavonoids are antioxidants found in *M. oleifera* with the ability to eliminate reactive oxygen species.

### 3.2. Polyphenols compositions of *M. oleifera* hydroethanolic and aqueous extracts

Data on the chromatographic characteristics (retention time, UV-Vis spectra in the maximum absorption, molecular ion, and main MS<sup>2</sup> fragments) and tentative identification of the phenolic compounds found in the hydroethanolic, infused and decocted extracts of *M. oleifera* are described in Table 2. Twenty-four phenolic compounds were found, being 19 glycosylated flavonol derivatives, 3 phenolic acids, and 2 flavan-3-ols. The phenolic composition of *M. oleifera* has been extensively studied by other authors (Makita, Chimuka, Steenkamp, Cukrowska, & Madala, 2016; Nouman et al., 2016; Ramabulana et al., 2016; Ziani et al., 2019); however, there are many compounds identified in the present work that, to the best of the author's knowledge, have never been previously identified in *M. oleifera*. Peaks 3, 6, 13, 14, 15, 18, 20, 21, and 22 were identified as (+)-catechin, (-)-epicatechin,

quercetin-3-O-rutinoside, apigenin-6-C-glucoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, kaempferol-3-O-glucoside, and isorhamnetin-3-O-glucoside, respectively, by comparing their retention time, UV-Vis spectra, and mass fragmentation patterns with those of the available commercial standards. Only three phenolic acids were tentatively identified, peaks 1/2 ([M-H]<sup>-</sup> at  $m/z$  337) and 4 ([M-H]<sup>-</sup> at  $m/z$  367), as *cis/trans* 3-O-*p*-coumaroylquinic acid and 3-O-feruloylquinic acid, respectively. Peak 1 presented a base peak at  $m/z$  191 (quinic acid) along with a peak at  $m/z$  163 (corresponding to the *p*-coumaroyl acid moiety); peak 2 presented the same chromatographic behaviour, leading to the respective identification of the *cis* and *trans* isomers of *p*-coumaroylquinic acid. These peaks (1/2 and 4) have been previously identified in the foliar parts of *M. oleifera* from South Africa, after being exposed to certain levels of radiation (Ramabulana et al., 2016).

The flavonoid was, without any doubt, the most abundant group of phenolic compounds identified in studied *M. oleifera* samples, with glycosylated derivatives of quercetin having a superior numerical expression to any other identified flavonoid aglycone. Peaks 10 ([M-H]<sup>-</sup> at  $m/z$  625), 16 ([M-H]<sup>-</sup> at  $m/z$  505), and 17/19 ([M-H]<sup>-</sup> at  $m/z$  549), tentatively identified as quercetin-O-dihexoside, quercetin-O-acetylhexoside and quercetin-malonylhexoside, respectively, have been previously identified in the leaves of *M. oleifera* from South Africa (Ramabulana et al., 2016), Pakistan (Nouman et al., 2016), and Namibia (Makita et al., 2016). Peak 5 presented a pseudomolecular ion [M-H]<sup>-</sup> at  $m/z$  711, and MS<sup>2</sup> fragments at  $m/z$  667 (loss of 44 u, carboxyl radical),  $m/z$  505 (loss of sinapoyl radical),  $m/z$  463 (loss of sinapoyl and acetyl radicals), and  $m/z$  301 (quercetin aglycone), which allowed the tentative identification as quercetin-acetylglucoside-sinapic acid. This peak has not been identified in *M. oleifera* samples, so its tentative identification was performed following the previously described by Medina et al. (2017) in *Passiflora edulis* shell, without numbering the oxygen atoms and radicals position since it was not possible to compare the abundance of each fragment. Peak 11, also a glycosylated derivative of quercetin, presented a pseudomolecular ion [M-H]<sup>-</sup> at  $m/z$  595, and MS<sup>2</sup> fragments at  $m/z$  463 and  $m/z$  301, corresponding to the loss of a pentosyl and hexosyl moieties, respectively. As peak 5, peak 11 was not previously identified in *M. oleifera* samples, so its tentative identification followed the previously described by Barros et al. (2013) in *Cistus ladanifer*. The second major flavonoid group was that of C-glycosylated apigenin derivatives, represented by peaks 7 ([M-H]<sup>-</sup> at  $m/z$  593), 9 ([M-H]<sup>-</sup> at  $m/z$  593) and 12 ([M-H]<sup>-</sup> at  $m/z$  431), tentatively identified as apigenin-6,8-C-diglucoside, apigenin-O-hexoside-C-hexoside, and apigenin-C-hexoside, respectively, following the previously described by Truchado, Vit, Ferreres, and Tomas-Barberan (2011) and Qiao et al. (2011), being previously identified similar compounds in *M. oleifera* leaves (Nouman et al., 2016; Ramabulana et al., 2016). Kaempferol derivatives were also found; peak 8, tentatively identified as kaempferol-O-malonylhexoside, was previously reported in *M. oleifera* leaf samples by Makita et al. (2016), and peak 23, presenting a pseudomolecular ion [M-H]<sup>-</sup> at  $m/z$  695, was tentatively identified as kaempferol-O-malonyldihexoside, following the previously described by Sánchez-Salcedo et al. (2016) in *Morus* spp. leaves (to the best of the authors' knowledge, this peak has not been described previously in *M. oleifera*). Finally, peak 24, tentatively identified as isorhamnetin-O-malonylhexoside, was previously described in *M. oleifera* leaves by Ziani et al. (2019).

Data on the quantification of the phenolic compounds present in *M. oleifera* edible parts are presented in Table 3. The profile of phenolic compounds present in each group of *M. oleifera* samples was very different, quantitatively but also qualitatively, with very few similar compounds between samples, which could be explained by the different physiological function of the studied plant parts and/or different microenvironmental conditions in each sampling site, namely a wetter and more shaded environment at the Ponta Romana homegarden.

The hydroethanolic extracts prepared with flowers from Bissau

**Table 2**

Phenolic compounds identified in *M. oleifera* edible parts. It is presented the retention time (Rt), wavelengths of maximum absorption in the visible region ( $\lambda_{\text{max}}$ ), and mass spectral data.

Peak	Rt (min)	$\lambda_{\text{max}}$ (nm)	[M – H] <sup>+</sup> (m/z)	MS <sup>2</sup> (m/z)	Tentative identification	Reference/method used for quantification
1	6.19	311	337	191(8), 173(6), 163(100), 153(3), 119(5)	<i>cis</i> 3- <i>O</i> - <i>p</i> -Coumaroylquinic acid	Ramabulana et al. (2016)
2	7.09	311	337	191(8), 173(6), 163(100), 153(3), 119(5)	<i>trans</i> 3- <i>O</i> - <i>p</i> -Coumaroylquinic acid	Ramabulana et al. (2016)
3	7.11	280	289	245(25), 203(10), 137(31)	(+)-Catechin	Standard compound
4	7.16	323	367	193(100), 191(5), 173(5), 149(3), 134(8)	3- <i>O</i> -Feruloylquinic acid	Ramabulana et al. (2016)
5	8.6	256/268/351	711	667(52), 505(100), 463(37), 301(21)	Quercetin- <i>O</i> -acetylglucosyl-sinapic acid	Medina et al. (2017)
6	9.57	280	289	245(100), 205(52), 151(29), 137(37)	(-)-Epicatechin	Standard compound
7	9.97	322	593	575(11), 503(24), 473(100), 383 (12), 353(27)	Apigenin-6,8- <i>C</i> -diglucoside	Truchado et al. (2011)
8	12.59	342	695	651(53), 489(100), 447(28), 285(41)	Kaempferol- <i>O</i> -malonyldihexoside	Sánchez-Salcedo et al. (2016)
9	13.55	337	593	473(35), 431(100), 353(5), 311(62), 283(5)	Apigenin- <i>O</i> -hexoside- <i>C</i> -hexoside	Qiao et al. (2011)
10	15.05	359	625	301(100)	Quercetin- <i>O</i> -dihexoside	Nouman et al. (2016)
11	15.98	350	595	463(31), 301(100)	Quercetin- <i>O</i> -pentoside- <i>O</i> -hexoside	Barros et al. (2013)
12	16.51	334	431	413(5), 341(6), 311(100)	Apigenin- <i>C</i> -hexoside	Nouman et al. (2016)
13	17.77	354	609	301(100)	Quercetin-3- <i>O</i> -rutinoside	Standard compound
14	18.35	337	431	413(7), 341(26), 311(100)	Apigenin-6- <i>C</i> -glucoside	Standard compound
15	18.91	353	463	301(100)	Quercetin-3- <i>O</i> -glucoside	Standard compound
16	20.19	353	505	463(30), 301(100)	Quercetin- <i>O</i> -acetylhexoside	Ramabulana et al. (2016)
17	20.21	352	549	505(12), 463(22), 301(100)	Quercetin- <i>O</i> -malonylhexoside	Makita et al. (2016)
18	21.06	347	593	285(100)	Kaempferol-3- <i>O</i> -rutinoside	Standard compound
19	22.06	350	549	505(72), 463(27), 301(100)	Quercetin- <i>O</i> -malonylhexoside	Makita et al. (2016)
20	22.07	353	623	315(100)	Isorhamnetin-3- <i>O</i> -rutinoside	Standard compound
21	22.39	346	447	285(100)	Kaempferol-3- <i>O</i> -glucoside	Standard compound
22	23.36	352	477	315(100)	Isorhamnetin-3- <i>O</i> -glucoside	Standard compound
23	24.62	346	533	489(89), 447(10), 285(100)	Kaempferol- <i>O</i> -malonylhexoside	Makita et al. (2016)
24	25.92	353	563	519(88), 315(100)	Isorhamnetin- <i>O</i> -malonylhexoside	Ziani et al. (2019)

presented the highest total concentration of phenolic compounds,  $14.7 \pm 0.1$  mg/g of extract, followed by the Quinhamel flower hydroethanolic extract, with  $13.8 \pm 0.1$  mg/g of extract. The seed samples were the only ones presenting flavan-3-ols derivatives, representing the major group of phenolics within this group. Another information that is important to highlight is the fact that the decoction prepared with the Quinhamel fruit sample had no phenolic compounds. Although an aqueous preparation such as decoction can lead to the thermal degradation of compounds, the absence of compounds may be related to the sample itself, since the hydroethanolic extract of this sample also had the lowest total concentration of phenolic compounds ( $0.765 \pm 0.001$  mg/g extract) within the corresponding group of samples.

Despite the very different phenolic profile, the most abundant phenolic compound (apart from seeds samples) was peak 1 (*cis* 3-*O*-*p*-coumaroylquinic acid), which did not produce an effect of higher concentration of phenolic acids, since it was the group of flavonoids that stood out (less in the Quinhamel flower hydroethanolic extract). These results are in accordance with the described by Ziani et al. (2019) and Nouman et al. (2016) in *M. oleifera* leaves, in which they revealed total concentrations of flavonoids of up to 30 mg/g extract and 2.98 mg/g extract, respectively.

### 3.3. Bioactive properties of *M. Oleifera* hydroethanolic and aqueous extracts

To evaluate the bioactive properties of the different *M. oleifera* edible parts, hydroethanolic, infused and decocted extracts were prepared according to traditional uses and applications. Fruits are traditionally prepared as a culinary vegetable, stewed in curries and soups. In India and Bangladesh, fruits are usually prepared by boiling pods to the desired level of tenderness in a mixture of coconut milk and spices (Lim, 2014). Therefore, only hydroethanolic and decocted extracts were prepared in this study with the fruit samples. On the other hand, seeds and flowers were used to prepare hydroethanolic, infused and decocted extracts. Traditionally, mature seeds are fried and eaten like peanuts in Nigeria and added to sauces for their bitter taste. In Pakistan are used to

prepare *M. oleifera* seed tea infusions (Ilyas et al., 2015) and in India seed decoctions (Dhakar et al., 2011). The flowers are cooked and consumed either mixed with other foods or fried in batter, butter or oil. In West Bengal and Bangladesh, these are usually cooked with green peas and potato, while in Africa are eaten as a vegetable, added to sauces or used to make infusions (Lim, 2014).

#### 3.3.1. Antioxidant activity

Two *in vitro* cell-based assays were used to measure the antioxidant activity of the hydroethanolic, infused and decocted extracts of the different *M. oleifera* parts (Table 4). These assays evaluate the extract ability to inhibit the formation of thiobarbituric acid reactive substances (TBARS) and the oxidative haemolysis (OxHLIA) using porcine brain tissues and erythrocytes as oxidizable biological substrates, respectively. As can be observed in Table 4, in the TBARS assay, significant differences were found between the three plant parts and between the extraction methods. The hydroethanolic extracts showed the lowest EC<sub>50</sub> values, thus translating a greater capacity to inhibit the TBARS formation than the aqueous extracts.

This result could be justified by the greater efficiency of the hydroethanolic mixture in extracting phenolic compounds and other antioxidants (Padayachee & Baijnath, 2020). In the OxHLIA assay, the sheep erythrocytes were subjected to the haemolytic action of both hydrophilic and lipophilic radicals generated in *in vitro* by the thermal decomposition of the free-radical initiator AAPH and as a consequence of the initial attack, respectively. By observing the data presented in Table 4, it can be noticed that infusions prepared with seed and flower samples from Bissau showed the best results, with IC<sub>50</sub> values lower than those of the trolox, the water-soluble analog of vitamin E used as a positive control. Interestingly, the hydroethanolic extracts did not show any antihemolytic effect. In a previous study, Pakade, Cukrowska, and Chimuka (2013) compared the antioxidant activity of *M. oleifera* leaves and flowers to that of several vegetables from South Africa, including spinach, cauliflower, broccoli, cabbage, and peas, and reported a total flavonoid content in *M. oleifera* three times higher than that quantified in the others plant foods, thus concluded that *M. oleifera* is a better source of antioxidants.

**Table 3**  
Content (mg/g extract) of the phenolic compounds identified in hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

Peak	Seeds						Flowers					
	Quinhamel			Bissau			Quinhamel			Bissau		
	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec
1	nd	nd	nd	nd	nd	nd	4.7 ± 0.1 <sup>a</sup>	nd	nd	1.214 ± 0.01 <sup>e</sup>	nd	nd
2	nd	nd	nd	nd	nd	nd	0.39 ± 0.01 <sup>a</sup>	nd	nd	nd	nd	nd
3	0.178 ± 0.002 <sup>a</sup>	0.035 ± 0.001 <sup>c</sup>	0.10 ± 0.01 <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5	nd	nd	nd	nd	nd	nd	0.23 ± 0.01 <sup>a</sup>	nd	nd	0.092 ± 0.001 <sup>c</sup>	nd	nd
6	0.44 ± 0.02 <sup>a</sup>	0.081 ± 0.004 <sup>d</sup>	0.07 ± 0.01 <sup>e</sup>	0.10 ± 0.02 <sup>c</sup>	0.28 ± 0.01 <sup>b</sup>	0.29 ± 0.01 <sup>b</sup>	nd	nd	nd	0.051 ± 0.003 <sup>f</sup>	nd	nd
7	nd	nd	nd	nd	nd	nd	0.254 ± 0.001 <sup>c</sup>	nd	nd	0.098 ± 0.01 <sup>c</sup>	nd	nd
8	nd	nd	nd	nd	nd	nd	0.262 ± 0.001 <sup>a</sup>	nd	nd	nd	nd	nd
9	0.08 ± 0.01 <sup>a</sup>	0.024 ± 0.004 <sup>c</sup>	0.072 ± 0.002 <sup>b</sup>	nd	nd	nd	tr	nd	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	0.061 ± 0.001	nd	nd	nd	nd	nd
11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	0.05 ± 0.02 <sup>a</sup>	0.008 ± 0.001 <sup>c</sup>	0.010 ± 0.002 <sup>b</sup>	nd	nd	nd	0.239 ± 0.001 <sup>c</sup>	nd	nd	0.2 ± 0.1 <sup>d</sup>	nd	nd
13	nd	nd	nd	nd	nd	nd	0.47 ± 0.01 <sup>a</sup>	nd	nd	0.109 ± 0.003 <sup>c</sup>	nd	nd
14	0.041 ± 0.001 <sup>e</sup>	0.050 ± 0.001 <sup>d</sup>	0.003 ± 0.0001 <sup>f</sup>	nd	nd	nd	0.678 ± 0.002 <sup>a</sup>	nd	nd	0.100 ± 0.001 <sup>g</sup>	nd	nd
15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	nd	nd	nd	nd	nd	nd	2.2 ± 0.1 <sup>b</sup>	nd	nd	nd	nd	nd
17	nd	nd	nd	nd	nd	nd	0.49 ± 0.02 <sup>d</sup>	nd	nd	0.48 ± 0.01 <sup>d</sup>	nd	nd
18	nd	nd	nd	nd	nd	nd	0.75 ± 0.02 <sup>a</sup>	nd	nd	0.184 ± 0.01 <sup>c</sup>	nd	nd
19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
20	nd	nd	nd	nd	nd	nd	0.32 ± 0.01 <sup>b</sup>	nd	nd	0.107 ± 0.001 <sup>d</sup>	nd	nd
21	nd	nd	nd	nd	nd	nd	1.3 ± 0.1 <sup>a</sup>	nd	nd	0.093 ± 0.002 <sup>d</sup>	nd	nd
22	nd	nd	nd	nd	nd	nd	0.83 ± 0.01 <sup>a</sup>	nd	nd	0.19 ± 0.01 <sup>e</sup>	nd	nd
23	nd	nd	nd	nd	nd	nd	0.67 ± 0.02 <sup>a</sup>	nd	nd	0.176 ± 0.001 <sup>e</sup>	nd	nd
24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TPA	nd	nd	nd	nd	nd	nd	5.1 ± 0.1 <sup>a</sup>	nd	nd	1.214 ± 0.01 <sup>e</sup>	nd	nd
TPFO	0.62 ± 0.02 <sup>a</sup>	0.116 ± 0.001 <sup>d</sup>	0.173 ± 0.001 <sup>c</sup>	0.10 ± 0.02 <sup>e</sup>	0.28 ± 0.01 <sup>b</sup>	0.29 ± 0.01 <sup>b</sup>	nd	nd	nd	1.9 ± 0.1 <sup>e</sup>	nd	nd
TF	0.17 ± 0.01 <sup>f</sup>	0.037 ± 0.001 <sup>j</sup>	0.081 ± 0.002 <sup>h</sup>	nd	nd	nd	8.76 ± 0.03 <sup>b</sup>	nd	nd	3.1 ± 0.1 <sup>d</sup>	nd	nd
TPC	0.79 ± 0.02 <sup>g</sup>	0.152 ± 0.002 <sup>i</sup>	0.254 ± 0.003 <sup>j</sup>	0.10 ± 0.02 <sup>m</sup>	0.28 ± 0.01 <sup>i</sup>	0.29 ± 0.01 <sup>i</sup>	13.8 ± 0.1 <sup>b</sup>	nd	nd	nd	nd	nd
Peak	Fruits						Fruits					
	Quinhamel			Bissau			Quinhamel			Bissau		
	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf
1	1.443 ± 0.003 <sup>d</sup>	3.86 ± 0.02 <sup>b</sup>	1.93 ± 0.03 <sup>c</sup>	0.61 ± 0.01 <sup>f</sup>	nd	nd	nd	0.50 ± 0.01 <sup>g</sup>	nd	0.20 ± 0.01 <sup>h</sup>	nd	nd
2	nd	0.471 ± 0.00 <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	nd	nd	nd	nd	nd	nd	nd	0.08 ± 0.01 <sup>a</sup>	nd	0.030 ± 0.003 <sup>-*</sup>	nd	nd
5	0.14 ± 0.02 <sup>b</sup>	0.020 ± 0.002 <sup>d</sup>	0.02 ± 0.01 <sup>d</sup>	tr	nd	nd	nd	nd	nd	nd	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	0.161 ± 0.001 <sup>d</sup>	0.69 ± 0.02 <sup>a</sup>	0.39 ± 0.01 <sup>b</sup>	0.15 ± 0.01 <sup>e</sup>	nd	nd	nd	nd	nd	nd	nd	nd
8	0.15 ± 0.04 <sup>b</sup>	tr	nd	tr	nd	nd	nd	nd	nd	nd	nd	nd
9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	tr	tr	nd	tr	nd	nd	nd	nd	nd	nd	nd	nd
11	tr	tr	nd	tr	nd	nd	nd	nd	nd	nd	nd	nd
12	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	0.136 ± 0.001 <sup>f</sup>	2.44 ± 0.01 <sup>a</sup>	0.55 ± 0.02 <sup>b</sup>	0.19 ± 0.01 <sup>d</sup>	nd	nd	nd	0.16 ± 0.01 <sup>e</sup>	nd	tr	nd	nd

(continued on next page)

Table 3 (continued)

Peak	Flowers			Fruits		
	Quinhamel			Quinhamel		
	Dec	HyEth	Inf	Dec	HyEth	Dec
14	0.35 ± 0.02 <sup>b</sup>	nd	nd	nd	nd	nd
15	0.159 ± 0.001 <sup>d</sup>	0.55 ± 0.01 <sup>b</sup>	0.326 ± 0.001 <sup>c</sup>	0.126 ± 0.001 <sup>f</sup>	0.20 ± 0.03 <sup>e</sup>	nd
16	nd	nd	nd	0.156 ± 0.001	nd	nd
17	nd	3.4 ± 0.1 <sup>a</sup>	0.95 ± 0.01 <sup>c</sup>	nd	nd	nd
18	1.13 ± 0.03 <sup>b</sup>	1.31 ± 0.01 <sup>a</sup>	0.53 ± 0.02 <sup>c</sup>	0.27 ± 0.01 <sup>d</sup>	0.18 ± 0.03 <sup>f</sup>	nd
19	0.71 ± 0.01 <sup>b</sup>	0.10 ± 0.01 <sup>d</sup>	0.073 ± 0.004 <sup>e</sup>	0.22 ± 0.01 <sup>e</sup>	nd	nd
20	nd	nd	nd	0.020 ± 0.002 <sup>f</sup>	nd	nd
21	0.17 ± 0.01 <sup>c</sup>	0.38 ± 0.01 <sup>a</sup>	nd	nd	0.214 ± 0.001	nd
22	0.14 ± 0.01 <sup>c</sup>	0.247 ± 0.001 <sup>b</sup>	nd	0.11 ± 0.01 <sup>d</sup>	nd	nd
23	0.39 ± 0.01 <sup>c</sup>	0.76 ± 0.01 <sup>b</sup>	0.254 ± 0.003 <sup>d</sup>	0.118 ± 0.001 <sup>c</sup>	nd	nd
24	0.31 ± 0.01 <sup>c</sup>	0.451 ± 0.004 <sup>b</sup>	0.21 ± 0.01 <sup>d</sup>	0.11 ± 0.01 <sup>f</sup>	0.12 ± 0.03 <sup>f</sup>	nd
TPA	1.443 ± 0.003 <sup>d</sup>	4.33 ± 0.02 <sup>b</sup>	1.929 ± 0.003 <sup>c</sup>	0.084 ± 0.003 <sup>g</sup>	0.20 ± 0.03 <sup>d</sup>	nd
TF3O	nd	nd	nd	0.61 ± 0.01 <sup>f</sup>	0.579 ± 0.002 <sup>g</sup>	0.231 ± 0.00- 3 <sup>h</sup>
TF	3.94 ± 0.04 <sup>c</sup>	10.3 ± 0.1 <sup>a</sup>	3.30 ± 0.04 <sup>d</sup>	nd	nd	nd
TPC	5.4 ± 0.1 <sup>c</sup>	14.7 ± 0.1 <sup>a</sup>	5.23 ± 0.04 <sup>c</sup>	1.10 ± 0.02 <sup>g</sup>	1.09 ± 0.02 <sup>g</sup>	0.231 ± 0.00- 3 <sup>k</sup>
				1.71 ± 0.03 <sup>e</sup>	1.66 ± 0.02 <sup>f</sup>	

nd- not detected; tr- trace amounts; nq - not quantifiable; HyEth - Hydroethanolic extract; Inf - Infusion preparation; Dec- Decoction preparation. TPA- Total Phenolic Acids; TF3O- Total Flavan-3-ol; TF - Total Flavonoids; TPC- Total Phenolic Compounds. Standard calibration curves: quercetin-3-O-rutinoside ( $y = 13343x + 76751$ ,  $R^2 = 0.9998$ , limit of detection (LOD) = 0.18 µg/mL and limit of quantitation (LOQ) = 0.65 µg/mL, peaks 5, 8, 10, 11, 13, 18, and 20); apigenin-6-C-glucoside ( $y = 107025x + 61531$ ,  $R^2 = 0.9989$ , LOD = 0.19 µg/mL and LOQ = 0.63 µg/mL, peaks 7, 9, 12, and 14); quercetin-3-O-glucoside ( $y = 34843x - 160173$ ,  $R^2 = 0.9998$ , LOD = 0.21 µg/mL and LOQ = 0.71 µg/mL, peaks 15, 16, 17, 19, 21, 22, 23, and 24); ferulic acid ( $y = 633126x - 185462$ ,  $R^2 = 0.999$ , LOD = 0.20 µg/mL and LOQ = 1.01 µg/mL, peak 4); (+)-catechin ( $y = 84950x - 23200$ ,  $R^2 = 1$ , LOD = 0.17 µg/mL and LOQ = 0.68 µg/mL, peaks 3 and 6); and *p*-coumaric acid ( $y = 301950x + 6966.7$ ,  $R^2 = 0.9999$ , LOD = 0.68 µg/mL and LOQ = 1.61 µg/mL, peaks 1 and 2). In each row different letters mean statistically significant differences ( $p < 0.05$ ). \*Mean statistical differences obtained by *t*-Student test.



### 3.3.2. NO-production inhibition activity

The NO-production inhibition (or anti-inflammatory) activity of the tested *M. oleifera* extracts was assessed based on the NO-production inhibition activity and the results are presented in Table 4. The extracts prepared with the seed samples from both locations were able to reduce the production of NO by LPS-stimulated murine macrophages. This result followed the same trend observed for the TBARS formation inhibition assay, with the hydroethanolic preparations showing the best results. However, flower and fruit extracts did not reveal anti-inflammatory activity at the tested concentrations. In previous studies, Minaïyan, Asghari, Taheri, Saeidi, and Nasr-Esfahani (2014) showed that hydroalcoholic seed extracts are effective in the treatment of experimental colitis and associated this effect with the major bioactive biophenols and flavonoids (Minaïyan et al., 2014). In turn, Jaja-Chimedza et al. (2017) connected the anti-inflammatory and antioxidant properties of *M. oleifera* seeds to the presence of isothiocyanates. Accordingly Padayachee and Baijnath (2020), infusions of *M. oleifera* leaves, seeds, flowers, roots, and bark display anti-inflammatory activity. Alhakmani, Kumar, and Khan (2013) also attributed anti-inflammatory effects to the *M. oleifera* flower extract, which supports the traditional use of this preparation in Oman and other Asian countries.

### 3.3.3. Cytotoxicity to tumour and non-tumour cells

Considering the described uses of the different parts of *M. oleifera* in traditional medicine, the prepared extracts were also tested for their cytotoxicity for tumour and non-tumour cell lines. The performed sulforhodamine B assay allows to evaluate the effect of the extracts on cell proliferation (Ziani et al., 2019). Therefore, GI<sub>50</sub> values translate the extract concentration providing 50% of cell growth inhibition. As presented in Table 4, the hydroethanolic extracts of seed and flower samples originated the lower GI<sub>50</sub> values, thus translating a higher activity than the aqueous extracts against HeLa (cervical), HepG2 (hepatocellular), MCF-7 (breast) and NCI-H460 (lung) tumour cells. Among the hydroethanolic extracts, those prepared with seeds were more effective against the HepG2 cell line, regardless of the geographic origin of the samples (with GI<sub>50</sub> of 82 ± 5 – 95 ± 2 µg/mL), while those prepared with flowers were more cytotoxic to breast MCF-7 cells (with GI<sub>50</sub> of 163 ± 5 – 187 ± 10 µg/mL). For seeds, the decoctions proved to be the least cytotoxic preparations for the tested cell lines (given the higher GI<sub>50</sub> values), which is in line with the results obtained with the OxHLIA assay (where they also had the highest IC<sub>50</sub> values). The aqueous flower extracts were not cytotoxic at the tested concentrations, nor any of those prepared with the fruits.

In previous studies, Jung (2014) found that aqueous *M. oleifera* leaf extracts are able to reduce the proliferation and invasion of cancer cells by inducing apoptosis, inhibiting the tumour cell growth and decreasing the level of internal reactive oxygen species in human lung cancer cells. Al-Asmari et al. (2015) evaluated the anticancer properties of *M. oleifera* leaf, bark and seed extracts against breast (MDA-MB-231) and colorectal (HCT-8) cancer cells and obtained remarkable anticancer activities with the leaf and bark extracts, while the seed extract showed less activity. It has also been reported that the flavonoids quercetin and kaempferol present in *M. oleifera* extracts may act as potential chemopreventive agents, being able to reduce the proliferation of human carcinoma through the induction of *in vitro* apoptosis (Padayachee & Baijnath, 2020). In addition, the presence of these and other antioxidants in *M. oleifera* allows to reduce oxidative stress and, consequently, help prevent the development of cancer. Among the metabolites with antioxidant activity found in *M. oleifera* are flavonoids, phenolic acids, saponins, tannins, β-carotene, and terpenoids (Singh et al., 2020).

Table 4 also shows that, with the exception of the hydroethanolic seed extracts, no other extract was cytotoxic to the non-tumour PLP2 cells at the tested concentrations. This toxicity of the hydroethanolic seed extracts to porcine liver primary cells may somehow justify the

absence of antihemolytic activity in the OxHLIA assay, since the erythrocytes may have been rapidly lysed due to the cytotoxic effect of these hydroalcoholic preparations.

In many countries, *M. oleifera* seed powder is used to purify water on aquaculture farms due to its coagulation properties. Nevertheless, the application of a large amount of this ingredient in aquaculture ponds leads to fish mortality due to the presence of toxic or antinutritional compounds. The seed powder toxicity has already been observed in guppies (*Poecilia reticulata*), Nile tilapia (*Oreochromis niloticus*), protozoa (*Tetrahymena pyriformis*), and bacteria (*Escherichia coli*) (Kavitha, Ramesh, Kumaran, & Lakshmi, 2012). Regarding ethanolic and aqueous extracts of both *M. oleifera* fruits and leaves, Luqman, Srivastava, Kumar, Maurya, and Chanda (2011) showed that these are well tolerated by experimental animals without toxicity of the extracts up to a dose of 100 mg/kg of body weight. The aqueous and hydroethanolic extracts of *M. oleifera* flowers have also been described as having a significant hepatoprotective effect, which may be due to the presence of quercetin, a well-known flavonoid with hepatoprotective activity (Upadhyay et al., 2015). Furthermore, Singh et al. (2020) described that alcoholic and aqueous extracts from flowers and roots of *M. oleifera* act as hepatoprotectors against the effect of acetaminophen (a drug used to treat pain and fever) by decreasing the level of serum enzymatic markers and bilirubin levels.

### 3.3.4. Antimicrobial activity

The results of the antimicrobial activity of *M. oleifera* extracts are presented in Table 5. All the extracts had significant antimicrobial effects against the tested bacteria and fungi. The MIC and MBC values obtained for *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*, as well as for *Enterobacter cloacae* and *Salmonella* Typhimurium, were comparable to those of streptomycin and ampicillin, the antibiotics used as positive controls, thus translating a similar bacteriostatic and bactericidal activity. In general, decoctions were the most effective preparations against the tested bacteria and, in the case of seeds, it is also worth noting the higher activity of the hydroethanolic and infused extracts prepared with seeds from Quinhamel and the decocted extracts made with seed from Bissau. The antimicrobial activity of *M. oleifera* leaf, root, bark and seed extracts against bacteria, yeasts, dermatophytes, and helminths pathogenic to human was previously investigated by Upadhyay et al. (2015), which verified that the seed aqueous extract inhibits the growth of *Pseudomonas aeruginosa* and *S. aureus*. According to previous reports, the antimicrobial activity of *M. oleifera* seed powder is conferred by a short cationic protein (Singh et al., 2020), as well as by saponins, tannins, phenolics, and alkaloids (Padayachee & Baijnath, 2020).

The antifungal activity of the tested *M. oleifera* extracts resulted in MIC and MBC values lower or similar to those of the positive controls ketoconazole and bifonazole (Table 5). The antifungal activity of aqueous leaf extracts of *M. oleifera* was previously confirmed by Padayachee and Baijnath (2020) against *Penicillium* spp., while the ethanolic extract also inhibited *Candida albicans*, *Penicillium* spp., and *Mucor* spp. The phytochemical screening of this plant part revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, and cardiac glycosides, which may act as natural antimicrobials (Padayachee & Baijnath, 2020).

## 4. Conclusion

The results of the present study highlighted the nutritional quality of *M. oleifera* fruits, seeds and flowers from Bissau and Quinhamel and the bioactive potential of their herbal preparations. These edible and medicinal matrices stood out not only with high nutritional value, but also for their potential to be used in food fortification and in the development of new functional foods, nutraceuticals and pharmaceutical formulations. *M. oleifera* is a natural resource to be valorised by underprivileged population facing poverty and malnutrition issues, but

**Table 4**  
Antioxidant, anti-inflammatory and cytotoxic activities of hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

	Seeds			Flowers			Fruits			Student's t-test	p-value	Student's t-test	p-value
	Quinhamel	Bissau	p-value	Quinhamel	Bissau	p-value	Quinhamel	Bissau	p-value				
Antioxidant activity*													
TBARS (IC <sub>50</sub> , mg/mL)	Hydroethanolic	0.09 ± 0.01c	0.09 ± 0.01c	0.228	0.06 ± 0.01c	0.07 ± 0.01c	0.008	0.15 ± 0.01b	0.14 ± 0.01b	0.471			
	Infusion	0.42 ± 0.01b	0.92 ± 0.01a	< 0.001	1.23 ± 0.02a	0.99 ± 0.01a	< 0.001	np	np	-			
	Decoction	0.51 ± 0.02a	0.82 ± 0.02b	< 0.001	1.06 ± 0.06b	0.85 ± 0.04b	< 0.001	1.56 ± 0.02a	1.49 ± 0.05a	0.015			
OxHLIA (IC <sub>50</sub> , µg/mL)	Hydroethanolic	na	na	-	na	na	-	na	na	-			
	Infusion	5.1 ± 0.1b	2.4 ± 0.2b	< 0.001	17.0 ± 0.6b	2.8 ± 0.2b	< 0.001	np	np	-			
	Decoction	29 ± 3a	29 ± 2a	0.729	124 ± 2a	89 ± 2a	< 0.001	265 ± 7	55 ± 3	< 0.001			
Δt = 60 min	Hydroethanolic	na	na	-	na	na	-	na	na	-			
	Infusion	10.1 ± 0.2b	8.1 ± 0.8b	0.023	29 ± 1b	7.8 ± 0.7b	< 0.001	np	np	-			
	Decoction	101 ± 4 a	109 ± 4 a	0.079	222 ± 2 a	160 ± 3 a	< 0.001	583 ± 26	126 ± 4	< 0.001			
Δt = 120 min													
Anti-inflammatory activity**	Hydroethanolic	208 ± 14c	180 ± 9c	0.015	> 400	> 400	-	> 400	> 400	-			
	Infusion	230 ± 9b	237 ± 6a	0.153	> 400	> 400	-	np	np	-			
	Decoction	248 ± 4a	230 ± 17b	0.006	> 400	> 400	-	> 400	> 400	-			
Cytotoxicity to tumour cells***													
HeLa (GI <sub>50</sub> , µg/mL)	Hydroethanolic	160 ± 8c	173 ± 6c	0.001	272 ± 6	300 ± 9	< 0.001	> 400	> 400	-			
	Infusion	201 ± 16b	225 ± 15b	0.272	> 400	> 400	-	np	np	-			
	Decoction	229 ± 3a	230 ± 17a	0.854	> 400	> 400	-	> 400	> 400	-			
HepG2 (GI <sub>50</sub> , µg/mL)	Hydroethanolic	95 ± 2c	82 ± 5b	0.060	184 ± 12	222 ± 19	< 0.001	> 400	> 400	-			
	Infusion	208 ± 7b	224 ± 14a	0.016	> 400	> 400	-	np	np	-			
	Decoction	254 ± 6a	224 ± 17a	< 0.001	> 400	> 400	-	> 400	> 400	-			
MCF-7 (GI <sub>50</sub> , µg/mL)	Hydroethanolic	167 ± 7c	180 ± 13b	0.001	163 ± 5	187 ± 10	< 0.001	> 400	> 400	-			
	Infusion	202 ± 8b	233 ± 5a	0.001	> 400	> 400	-	np	np	-			
	Decoction	251 ± 7a	232 ± 4a	0.004	> 400	> 400	-	> 400	> 400	-			
NCI-H460 (GI <sub>50</sub> , µg/mL)	Hydroethanolic	105 ± 10c	129 ± 15b	< 0.001	245 ± 9	271 ± 13	< 0.001	> 400	> 400	-			
	Infusion	232 ± 19b	239 ± 4a	0.414	> 400	> 400	-	np	np	-			
	Decoction	301 ± 10a	239 ± 6a	< 0.001	> 400	> 400	-	> 400	> 400	-			
Cytotoxicity to non-tumour cells***													
PLP2 (GI <sub>50</sub> , µg/mL)	Hydroethanolic	327 ± 8	347 ± 7	0.075	> 400	> 400	-	> 400	> 400	-			
	Infusion	> 400	> 400	-	> 400	> 400	-	np	np	-			
	Decoction	> 400	> 400	-	> 400	> 400	-	> 400	> 400	-			

na - no activity; np - not performed. \*IC<sub>50</sub> values translate the extract concentration providing 50% of antioxidant activity (TBARS assay) or required to keep 50% of the erythrocyte population intact for 60 and 120 min (OxHLIA assay). Trolox IC<sub>50</sub> values: 19.6 ± 0.7 µg/mL (OxHLIA, Δt 60 min), 41 ± 1 µg/mL (OxHLIA, Δt 120 min), and 23 µg/mL (TBARS). \*\*EC<sub>50</sub> values translate the extract concentration providing 50% of NO-production inhibition. Dexamethasone EC<sub>50</sub> value: 16 µg/mL. \*\*\*GI<sub>50</sub> values correspond to the extract concentration responsible for 50% of cell growth inhibition. Ellipticine GI<sub>50</sub> values: 3 µg/mL (PLP2), 1 µg/mL (MCF-7), 1 µg/mL (NCI-H460), 2 µg/mL (HepG2). In each column, different letters correspond to significant differences between extracts (p < 0.05).

**Table 5**  
Antibacterial and antifungal activity of hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

		Seeds		Flowers		Fruits		Positive controls	
		Quinhamel	Bissau	Bissau	Quinhamel	Quinhamel	Fruits Bissau	Streptomycin	Ampicillin
Antibacterial activity (mg/mL)		MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
<i>B. cereus</i>	Hydroethanolic	0.075/0.15	0.10/0.20	0.10/0.20	0.10/0.20	0.20/0.40	0.20/0.40	0.04/0.10	0.25/0.45
	Infusion	0.075/0.15	0.60/0.90	0.15/0.30	0.15/0.30	np	np		
	Decoction	0.075/0.15	0.037/0.075	0.075/0.30	0.075/0.30	0.20/0.30	0.15/0.30		
<i>S. aureus</i>	Hydroethanolic	0.10/0.20	0.25/0.50	0.30/0.60	0.30/0.60	0.20/0.40	0.20/0.40	0.10/0.20	0.25/0.40
	Infusion	0.15/0.30	0.50/0.90	0.30/0.60	0.45/0.60	np	np		
	Decoction	0.075/0.15	0.037/0.075	0.075/0.30	0.15/0.30	0.20/0.30	0.20/0.30		
<i>L. monocytogenes</i>	Hydroethanolic	0.10/0.20	0.45/0.90	0.10/0.20	0.10/0.20	0.10/0.20	0.10/0.20	0.20/0.30	0.40/0.50
	Infusion	0.10/0.15	0.60/0.90	0.15/0.30	0.15/0.30	np	np		
	Decoction	0.075/0.15	0.037/0.075	0.05/0.10	0.20/0.30	0.20/0.30	0.075/0.15		
<i>E. coli</i>	Hydroethanolic	0.10/0.20	0.10/0.20	0.075/0.15	0.10/0.20	0.10/0.25	0.10/0.20	0.20/0.30	0.40/0.50
	Infusion	0.10/0.15	0.15/0.30	0.075/0.15	0.10/0.15	np	np		
	Decoction	0.05/0.15	0.037/0.075	0.10/0.15	0.20/0.30	0.075/0.15	0.10/0.15		
<i>E. cloacae</i>	Hydroethanolic	0.10/0.20	0.30/0.60	0.30/0.60	0.50/1.00	0.30/0.60	0.25/0.50	0.20/0.30	0.25/0.50
	Infusion	0.15/0.30	0.90/1.20	0.30/0.60	0.40/0.90	np	np		
	Decoction	0.05/0.15	0.037/0.075	0.075/0.15	0.20/0.30	0.075/0.15	0.10/0.15		
<i>S. Typhimurium</i>	Hydroethanolic	0.10/0.20	0.30/0.60	0.10/0.15	0.30/0.60	0.25/0.50	0.15/0.30	0.20/0.30	0.75/1.20
	Infusion	0.15/0.30	0.30/0.90	0.15/0.30	0.45/0.60	np	np		
	Decoction	0.037/0.075	0.018/0.075	0.25/0.60	0.25/0.60	0.20/0.30	0.075/0.15		
Antifungal activity (mg/mL)								Ketoconazole	Bifonazole
		MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC
<i>A. fumigatus</i>	Hydroethanolic	0.075/0.15	0.05/0.075	0.25/0.50	0.20/0.40	0.10/0.20	0.10/0.20	0.25/0.50	0.15/0.20
	Infusion	0.075/0.15	0.05/0.10	0.30/0.60	0.075/0.15	np	np		
	Decoction	0.018/0.037	0.075/0.15	0.018/0.037	0.075/0.15	0.075/0.15	0.075/0.15		
<i>A. ochraceus</i>	Hydroethanolic	0.075/0.15	0.075/0.15	0.015/0.030	0.075/0.15	0.10/0.20	0.10/0.20	0.20/0.50	0.10/0.20
	Infusion	0.037/0.075	0.037/0.075	0.075/0.15	0.037/0.075	np	np		
	Decoction	0.037/0.075	0.037/0.075	0.018/0.037	0.037/0.75	0.037/0.075	0.037/0.075		
<i>A. niger</i>	Hydroethanolic	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	0.30/0.60	0.30/0.60	0.20/0.50	0.15/0.20
	Infusion	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	np	np		
	Decoction	0.075/0.15	0.075/0.15	0.075/0.15	0.037/0.075	0.075/0.15	0.037/0.075		
<i>P. funiculosum</i>	Hydroethanolic	0.037/0.075	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	0.15/0.30	0.20/0.50	0.20/0.25
	Infusion	0.037/0.075	0.075/0.15	0.05/0.10	0.15/0.30	np	np		
	Decoction	0.037/0.075	0.037/0.075	0.037/0.075	0.075/0.15	0.075/0.30	0.037/0.075		
<i>P. ochrochloron</i>	Hydroethanolic	0.037/0.075	0.037/0.075	0.10/0.20	0.15/0.60	0.45/0.90	0.60/1.20	2.50/3.50	0.20/0.25
	Infusion	0.10/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np		
	Decoction	0.075/0.15	0.075/0.15	0.075/0.15	0.30/0.45	0.075/0.15	0.037/0.075		
<i>P. aurantioriseum</i>	Hydroethanolic	0.037/0.075	0.037/0.075	0.15/0.30	0.30/0.60	0.10/0.20	0.15/0.30	0.20/0.30	0.10/0.20
	Infusion	0.075/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np		
	Decoction	0.075/0.15	0.075/0.15	0.037/0.15	0.30/0.45	0.075/0.15	0.037/0.15		

MIC - minimum inhibitory concentrations; MBC - minimum bactericidal concentration; MFC - minimum fungicidal concentration; np - not performed.

also by other stockholders, specifically in underdeveloped and developing nations that have an insufficient technical resources.

#### CRediT authorship contribution statement

**Ângela Fernandes:** Methodology, Software, Validation, Investigation, Data curation, Writing - original draft, Writing - review & editing, Funding acquisition. **Aduca BanceSSI:** Writing - original draft. **José Pinela:** Software, Data curation, Writing - original draft, Writing - review & editing. **Maria Inês Dias:** Methodology, Software, Validation, Investigation, Data curation, Writing - original draft, Funding acquisition. **Ângela Liberal:** Methodology. **Ricardo C. Calhelha:** Methodology, Investigation, Data curation, Funding acquisition. **Ana Ćirić:** Methodology. **Marina Soković:** Supervision. **Luís Catarino:** Conceptualization, Visualization, Supervision. **Isabel C.F.R. Ferreira:** Writing - review & editing, Supervision, Project administration. **Lillian Barros:** Conceptualization, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.128229>.

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