

1 Fernandes, Ângela; Bancessi, Aducabe; Pinela, José; Dias, Maria Inês; Liberal, Ângela; Calhela,
2 Ricardo C.; Ciric, Ana; et al. "Nutritional and phytochemical profiles and biological activities of
3 Moringa oleifera Lam. edible parts from Guinea-Bissau (West Africa)". Food Chemistry 341 (2020):
4 128229. <http://dx.doi.org/10.1016/j.foodchem.2020.128229>.

5

6 Ângela FERNANDES ^{a,*}, Aducabe BANCESSI ^{b,c}, José PINELA ^a, Maria Inês DIAS ^a,
7 Ângela LIBERAL ^a, Ricardo C. CALHELHA ^a, Ana ĆIRIĆ ^c, Marina SOKOVIĆ ^d,
8 Luís CATARINO ^c, Isabel C.F.R. FERREIRA ^a, Lillian BARROS ^{a,*}

9

10 ^a *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus*
11 *de Santa Apolónia, 5300-253, Bragança, Portugal*

12 ^b *Nova School of Business and Economics, NOVA University of Lisbon, Campus de*
13 *Carcavelos, Rua da Holanda, n. 1, 2775-405 Carcavelos, Portugal*

14 ^c *Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculty of Sciences,*
15 *University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal*

16 ^d *Institute for Biological Research “Siniša Stanković” – National Institute of Republic of*
17 *Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia*

18

19

20 *Correspondence: L. Barros (lillian@ipb.pt); A. Fernandes (afeitor@ipb.pt).

21 **Abstract**

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

34

35

36 **Keywords:** *Moringa oleifera*; famine food; nutritional composition; phenolic profile;
37 antioxidant/anti-inflammatory activity; cytotoxicity.

38 1. Introduction

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

47 *M. oleifera* is one of the most auspicious plants used as a suitable alternative for preventing and
48 alleviating malnutrition challenges, especially hidden hunger health issues (Padayachee &
49 Baijnath, 2019). It is considered to be a “Miracle tree” or “Tree of life” due to the substantial
50 beneficial effects that it has on health, but also due to its potential use in water sanitation and
51 environmental conservation (Daba, 2016). *M. oleifera* preparations have been reported in the
52 scientific literature as having a wide range of pharmacological properties, including
53 antimicrobial, hypotensive, hypoglycemic, immunomodulatory, and anti-inflammatory
54 activities. In addition, all *M. oleifera* parts (including leaves, fruits, seeds, pods, and flowers)
55 have been used in traditional foods and dishes for human consumption (Daba, 2016).

56 The leaves and seeds are eaten fresh, powdered or cooked and contain a varied profile of
57 nutrients and health-promoting compounds, such as fatty acids, tocopherols, β -carotene, and
58 phenolic compounds. The fruits are fibrous and traditionally used to treat digestive problems
59 and prevent colon cancer. Flower extracts, in turn, are used in culinary preparations to enhance
60 the taste and colour of dishes (Padayachee & Baijnath, 2019; Ziani et al., 2019). These *M.*
61 *oleifera* organs are also known to be good sources of secondary metabolites, including
62 terpenoids, flavonoids, tannins, anthocyanins, and proanthocyanidins (Ajibade et al., 2013).

63 These bioactive compounds contribute to the therapeutic and medicinal properties of *M.*
64 *oleifera* and may justify its uses by the indigenous system of medicine in the treatment of
65 common ailments and disorders, such as anaemia, asthma, diarrhea, skin infections, headaches,
66 swelling, hysteria, cholera, scurvy, respiratory disorders, diabetes, cough, sore throat, and chest
67 congestion (Padayachee & Baijnath, 2019). Therefore, this edible medicinal plant appears as a
68 natural remedy easily accessible to populations in developing countries that need basic
69 healthcare, especially in areas where Western medicine is inaccessible or expensive
70 (Padayachee & Baijnath, 2019). Curiously, *M. oleifera* seed powder is used as a purifying agent
71 in the treatment of water, being able to eliminate pathogenic bacteria up to 99%, whereas fresh
72 leaves can be used to extract a juice used as a growth hormone (or soil fertilizer) able to increase
73 crop yields by 25-35% (Daba, 2016).

74 In Guinea-Bissau (West Africa), the awareness of local populations about the medicinal and
75 nutritional properties of *M. oleifera* has increased in the last years, where the trade of seeds and
76 dried and crushed leaves is under development. Despite this, the exploitation of the different
77 edible and medicinal parts of this plant in this country is far to reach their full potential
78 (BanceSSI et al., 2019). Therefore, due to the multiple traditional uses and applications of *M.*
79 *oleifera*, this study was performed to determine the detailed nutritional and chemical
80 composition (proximate constituents, free sugars, organic acids, tocopherols, fatty acids, and
81 phenolic compounds) of seed, flower and fruit samples collected in two distinct locations in
82 Guinea-Bissau using official methods of food analysis and advanced chromatographic
83 techniques. In addition, the antioxidant, anti-inflammatory, cytotoxic, and antimicrobial
84 activities of hydroethanolic, infused and decocted extracts prepared with the three *M. oleifera*
85 organs were assessed *in vitro* using different cellular assays and food-borne microorganisms.
86 In this way, it is intended to demonstrate and validate the food and medicinal potential of *M.*

87 *oleifera*, which can have a direct impact on the food security of local African populations and
88 be useful for the development of new functional foods and nutraceuticals.

89

90 **2. Material and methods**

91 *2.1. Sampling and samples preparation*

92 *M. oleifera* seeds, flowers and immature fruits (**Fig. 1**) were collected in early May 2019 in two
93 **locations** in Guinea-Bissau: Granja (11° 52'02''N; 15° 36'06''W), a state farm inside Bissau
94 urban area, and in a homegarden in Ponta Romana, Quinhamel, located in the countryside
95 (11°54'18''N; 15°49'45''W). **The two collecting sites are about 30 km apart and the soil and**
96 **climatic conditions in both sites are similar (ferralsols, rainfall c. 1500 mm per year). The main**
97 **differences are the urban vs. rural environment and the fact that in Granja the harvested trees**
98 **were isolated, with direct sunlight during most of the day and in Ponta Romana the samples**
99 **were taken from trees of a living fence in a homegarden, with less direct sunlight. The plant**
100 samples were then lyophilized (FreeZone 4.5, Labconco, MO, USA) and reduced to a fine
101 powder that was stored in well-sealed plastic bags at -20 °C **in the dark** until further analysis.

102

103 **2.2. Nutritional value and energy assessment**

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

109

110

111

112 2.3. Chromatographic analysis of free sugars, organic acids, fatty acids, and tocopherols

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

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

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

132 Tocopherols were determined using the internal standard (tocol, Matreya, **Pleasant Gap, PA,**
133 **USA**) method and the HPLC system (Smartline System 1000, Knauer, Berlin, Germany)
134 coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA) programmed for excitation
135 at 290 nm and emission at 330 nm, as previously described by Spréa et al. (2020). Data were

136 recorded and processed using Clarity 2.4 software and the results were given as mg per 100 g
137 dw.

138

139 2.4. Preparation of *M. oleifera* hydroethanolic and aqueous extracts

140 The *M. oleifera* seed, flower and immature fruit samples were prepared in hydroethanolic,
141 infused and decocted extracts to evaluate their composition in phenolic compounds and the *in*
142 *vitro* bioactive properties. These preparation/extraction methods were selected according to the
143 traditional uses of the different parts of the plant (Dhakar et al., 2011; Ilyas et al., 2015; Lim,
144 2014).

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

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

153 Only seeds and flowers were used to prepare infusions. The samples (2 g) were infused with
154 freshly boiled distilled water (100 mL), left aside for 5 min and subsequently filtered through
155 Whatman filter paper No 4. The resulting extracts were frozen and lyophilized (Adouni et al.,
156 2018).

157

158 2.5. HPLC-DAD-ESI/MSⁿ analysis of phenolic compounds

159 Phenolic compounds were analysed in hydroethanolic, infused and decocted extracts, which
160 were redissolved in ethanol/water (80:20, v/v) and water, respectively, to a final concentration

161 of 10 mg/mL and filtered using 0.22 µm disposable filter disks. The analysis was performed in
162 a HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, California, USA)
163 coupled with a diode-array detector (DAD, using 280 and 370 nm as preferred wavelengths)
164 and a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA,
165 USA) equipped with an electrospray ionization (ESI) source. Separation was made in a Waters
166 Spherisorb S3 ODS-2 C18 column (3 µm, 4.6 mm × 150 mm; Waters, Milford, MA, USA).
167 The operating conditions were previously described by Bessada, Barreira, Barros, Ferreira, and
168 Oliveira (2016), as well as the identification and quantification procedures. The results were
169 given as mg per g of extract.

170

171 2.6. Evaluation of bioactive properties *in vitro*

172 2.6.1. Antioxidant activity

173 Two cell-based assays were performed to measure the *in vitro* antioxidant activity of the
174 extracts (0.1563–5 mg/mL), following methodologies formerly described by Spréa et al. (2020)
175 and Lockowandt et al. (2019). The extracts capacity to inhibit the formation of thiobarbituric
176 acid reactive substances (TBARS) was assessed using porcine brain cell tissues as oxidizable
177 substrates, and the results were expressed as half maximal effective extract concentration (EC₅₀)
178 values (mg/mL). The oxidative haemolysis inhibition assay (OxHLIA) was performed to assess
179 the extracts capacity to protect sheep erythrocytes from the AAPH (2,2'-azobis(2-
180 methylpropionamide) dihydrochloride)-induced oxidative haemolysis. Half maximal
181 inhibitory concentration (IC₅₀) values (µg/mL) were calculated for time intervals (Δt) of 60 and
182 120 min and translate the extract concentration required to keep 50% of the erythrocyte
183 population intact for 60 and 120 min. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used
184 as a positive control.

185

186 *2.6.2. Nitric oxide (NO)-production inhibition activity*

187 The anti-inflammatory activity of the extracts (at concentrations up to 400 µg/mL) was assessed
188 based on the **nitric oxide (NO)** production by a lipopolysaccharide (LPS)-stimulated murine
189 macrophage (RAW 264.7) cell line. The NO production was quantified based on the nitrite
190 concentration using the Griess Reagent System kit containing sulphanilamide, *N*-1-
191 naphthylethylenediamine dihydrochloride and nitrite solutions, following a procedure
192 previously described by Corrêa et al. (2015). Dexamethasone (Sigma-Aldrich, St. Louis, MO,
193 USA) was used as a positive control, while no LPS was added in negative controls. The effect
194 of the tested extracts in NO basal levels was also assessed by performing the assay in the
195 absence of LPS. The results were expressed as IC₅₀ values (µg/mL), which correspond to the
196 extract concentration providing **50% of NO production inhibition**.

197

198 *2.6.3. Cytotoxic activity*

199 The extracts cytotoxicity was assessed by the sulforhodamine B (Sigma-Aldrich, St. Louis,
200 MO, USA) assay against four human tumour cell lines (acquired from Leibniz-Institut DSMZ),
201 namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa
202 (cervical carcinoma), and HepG2 (hepatocellular carcinoma), following a protocol previously
203 described by Spréa et al. (2020). Ellipticine (Sigma- Aldrich, St. Louis, MO, USA) was used
204 as a positive control. The same assay was also used to evaluate the hepatotoxicity of the extracts
205 against a non-tumour cell line (PLP2, porcine liver primary cells) obtained as described by
206 Spréa et al. (2020). **The extract concentration (µg/mL) causing 50% cell growth inhibition**
207 **(GI₅₀) was calculated and used to express the results.**

208

209

210

211 2.6.4. Antimicrobial activity

212 The extracts were redissolved in 5% dimethyl sulfoxide (DMSO) to a concentration of 10
213 mg/mL and further diluted. The microdilution method (Soković et al., 2010) was performed to
214 assess the antimicrobial activity against the Gram-negative bacteria *Escherichia coli* (ATCC
215 35210), *Salmonella* Typhimurium (ATCC 13311) and *Enterobacter cloacae* (ATCC 35030),
216 and the Gram-positive *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate)
217 and *Listeria monocytogenes* (NCTC 7973). The antifungal activity was assessed against
218 *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger*
219 (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC
220 9112), and *Penicillium aurantiogriseum* (food isolate) (Corrêa et al., 2015). The minimum
221 extract concentrations that completely inhibited bacterial growth (MICs) were determined by a
222 colorimetric microbial viability assay, and minimum bactericidal concentration (MBC) and
223 minimum fungicidal concentration (MFC) were also calculated. Streptomycin, ampicillin,
224 ketoconazole and bifonazole (Sigma-Aldrich, St. Louis, MO, USA) were used as positive
225 controls, and 5% DMSO was used as a negative control.

226

227 2.7. Statistical analysis

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

235

236 3. Results and discussion

237 3.1. Nutritional composition of *M. oleifera* edible parts

238 Since the plants composition is affected by different factors, such as the edaphoclimatic
239 conditions of the different **growing sites**, agricultural practices, harvesting period, and genetic
240 characteristics, among others (Iyda, Fernandes, Calhelha, et al., 2019), the studied samples of
241 *M. oleifera* were collected at two distinct locations in Guinea-Bissau. **Table 1** presents the
242 proximal composition of the *M. oleifera* seeds, flowers, and fruits collected in Quinhamel and
243 Bissau. Carbohydrates were found to be major constituents in all studied **samples**; the highest
244 levels were detected in the fruit (71.91 ± 0.04 and 79.6 ± 0.1 g/100 g dw) and the lowest in the
245 seeds (38.85 ± 0.03 and 41.2 ± 0.3 g/100 g dw in samples from Bissau and Quinhamel,
246 respectively). Proteins rank second with the seeds showing the higher levels (30.0 ± 0.6 –
247 31.88 ± 0.08 g/100 g dw), followed by the flower and the fruit. These last two **plant parts** also
248 had an interesting content of ash (total minerals), which ranged from 19.83 ± 0.01 to 21.3 ± 0.4
249 g/100 g dw. As expected, the seeds had a higher fat content (~ 26.3 g/100 g dw) than the other
250 two edible parts of *M. oleifera*. In addition, fruits collected in Quinhamel stood out with a
251 significantly higher fat content (4.3 ± 0.1 g/100 g dw) than those collected in Bissau (2.67 ± 0.06
252 g/100 g dw). The results obtained in this study are slightly lower than those previously reported
253 by **Gopalakrishnan, Doriya, and Kumar (2016)** and **Liang, Wang, Li, Chu, and Sun (2019)** for
254 the fat (38.67 and 39.12 g/100 g) and protein (35.97 and 40.34 g/100 g) contents in Indian *M.*
255 *oleifera* seeds, but were higher for carbohydrates (8.67 and 8.94 g/100 g).

256 Regarding the energy contribution, 100 g fruit and flower portions provide comparable values
257 (~ 390 – 396 kcal), while that of seeds were higher (~ 518 – 522 kcal) mainly due to the fat content.
258 According to previous reports, *M. oleifera* oil can accelerate wound healing (Liang et al., 2019)
259 and the seed protein fraction has potential to be used in surface water purification due to
260 coagulant effects (Baptista et al., 2017). Therefore, *M. oleifera* edible parts arise as interesting

261 possibilities for being exploited as raw materials for production of vegetable oil, protein-rich
262 foods and skincare products.

263 As shown in **Table 1**, the chromatographic analysis allowed to detect and quantify four free
264 sugars in the studied *M. oleifera* flowers and fruits, namely fructose, glucose, sucrose and
265 trehalose, while just glucose and fructose were found in the seeds. The highest levels were
266 quantified in the fruits (16.7 ± 0.1 – 18.8 ± 0.2 g/100 g fw), followed by the flowers (11.1 ± 0.1 –
267 12.0 ± 0.2 g/100 g fw) (**Fig. S1, supplementary material**) and lastly by the seeds with
268 significantly lower levels (1.32 ± 0.09 – 1.86 ± 0.06 g/100 g fw). It was also noted that the
269 quantitative sugar profile of the fruit and flower samples seemed to have been affected by their
270 different origin. These differences could be attributed to edaphoclimatic factors and some biotic
271 conditions that can affect biochemical and physiological processes involved in the plant sugars'
272 production (Ziani et al., 2019). In a previous study, Ziani et al. (2019) identified fructose,
273 glucose and sucrose in *M. oleifera* leaves from Algeria and reported a total free sugars content
274 of 3.82 g/100g dw. Upadhyay, Yadav, Mishra, Sharma, and Purohit (2015) described L-
275 arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, and D-xylose as the
276 predominant sugars in the purified whole-gum exudates of *M. oleifera*.

277 Regarding organic acids, the analysis allowed identifying oxalic, malic, ascorbic, citric, and
278 fumaric acids in flower and fruit samples from both locations (**Table 1**). Citric and malic acids
279 were the major compounds, while just traces of fumaric acid were detected. Fruits collected in
280 Bissau contained a higher level of ascorbic acid (0.65 ± 0.02 g/100 g fw) than those from
281 Quinhamel or the flower samples. The total organic acid contents ranged from 4.71 ± 0.02 –
282 5.75 ± 0.02 g/100 g fw in fruits to 5.85 ± 0.01 – 6.42 ± 0.01 g/100 g fw in flowers. In *M. oleifera*
283 seeds, ~ 10.5 g/100 g fw of oxalic acid were quantified (**Table 1**), about twice the total content
284 of organic acids found in the other two parts of the plant. Traces of fumaric acid were also
285 detected. It is known that plant foods with a high oxalic acid concentration should be consumed

286 moderately, because the high intake of oxalates may promote the formation of kidney stones,
287 irritation of the intestinal mucosa, and also interferes with calcium absorption (Iyda, Fernandes,
288 Ferreira, et al., 2019). To the best of the authors' knowledge, no data are available in the
289 literature regarding the organic acid composition of *M. oleifera* seeds, flowers or fruits. In
290 leaves, Ziani et al. (2019) already reported oxalic, malic and ascorbic acids.

291 The main fatty acids identified in the studied *M. oleifera* edible parts are also presented in **Table**
292 **1**, while the detailed profiles are shown in **Table S1** provided in Supplementary Material.

293 Twenty-one fatty acids were identified in the fruit and flower lipid fractions, while just 14 were
294 detected in the seed samples. The flower lipid fraction was mainly composed by unsaturated
295 fatty acid (SFA; ~41%, due to the contribution of C16:0, C22:0 and C18:0), followed by
296 polyunsaturated fatty acids (PUFA; 32.4±0.2 – 37.9±0.1 %), namely α -linolenic (C18:3n3) and
297 linoleic (C18:2n6) acids. *M. oleifera* fruits were abundant in monounsaturated fatty acids
298 (MUFA; 49.0±0.1 – 55.0±0.6 %), particularly those collected in Quinhamel homegardens, due
299 to the high contents of oleic acid (C18:1n9), followed by SFA (31.3±0.2– 33.4±0.5 %), which
300 predominated in the fruit samples from Bissau, given the high levels of palmitic (C16:0),
301 behenic (C22:0) and stearic (C18:0) acids. MUFA also predominated in the seed samples
302 (73.1±0.5 – 75.1±0.2 %), mostly C18:1n9 but also minor levels of eicosenoic (C20:1) and
303 palmitoleic (C16:1) acids. The SFA C16:0 and C22:0 were also detected in this plant part. In a
304 previous work, Zheng et al. (2019) studied the effects of soil drenching and foliar spraying of
305 boron on *M. oleifera* seed oil quality and reported C18:1 levels ranging from 64.24 to 71.17%,
306 a result comparable to that obtained in the present study (69.44±0.4 and 71.6±0.2% for seeds
307 from Bissau and Quinhamel, respectively). The lipid composition of *M. oleifera* seeds is greater
308 than that of soybean, which makes it nutritionally important and the refined seed oil is
309 acceptable to substitute the olive oil because of the presence of all the essential fatty acids in it
310 (Singh et al., 2019).

311 The tocopherols composition of the studied *M. oleifera* edible parts is shown in **Table 1**, where
312 it can be seen that α -tocopherol was the prevalent isoform in all samples, followed by δ -
313 tocopherol. **The flower samples showed the highest α -tocopherol concentrations, ranging from**
314 **17.22 \pm 0.09 to 18.90 \pm 0.01 mg/100 g dw (HPLC profile in Fig. S2, supplementary material).**
315 Fruit and seed samples revealed a total content of tocopherols ranging from 2.71 \pm 0.01 to
316 4.86 \pm 0.03 g/100 g dw and the samples collected in Bissau showed higher levels of these
317 lipophilic antioxidants. Singh et al. (2020) reported that tocopherols together with ascorbic acid,
318 carotenoids and flavonoids are **antioxidants** found in *M. oleifera* with the ability to eliminate
319 reactive oxygen species.

320

321 **3.2. Polyphenols compositions of *M. oleifera* hydroethanolic and aqueous extracts**

322 Data on the chromatographic characteristics (retention time, UV-Vis **spectra** in the maximum
323 absorption, molecular ion, and main MS² fragments) and tentative identification of the phenolic
324 compounds found in the hydroethanolic, infused and decocted extracts of *M. oleifera* are
325 described in **Table 2**. Twenty-four phenolic compounds were found, being 19 glycosylated
326 **flavonol derivatives**, 3 phenolic acids, and 2 flavan-3-ols. The phenolic composition of *M.*
327 *oleifera* has been extensively studied by other authors (Makita et al., 2016; Nouman et al., 2016;
328 Ramabulana et al., 2016; Ziani et al., 2019); however, there are many compounds identified in
329 the present **work** that, **to the best of the** author's knowledge, have never been previously
330 identified in *M. oleifera*. Peaks **3, 6, 13, 14, 15, 18, 20, 21, and 22** were identified as (+)-
331 catechin, (-)-epicatechin, quercetin-3-*O*-rutinoside, apigenin-6-*C*-glucoside, quercetin-3-*O*-
332 glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside,
333 and isorhamnetin-3-*O*-glucoside, respectively, by **comparing** their retention time, **UV-Vis**
334 **spectra**, and mass fragmentation patterns **with those of the** available commercial standards.
335 Only three phenolic acids were tentatively identified, peaks **1/2** ([M-H]⁻ at *m/z* 337) and **4** ([M-

336 $\text{H}]^-$ at m/z 367), as *cis/trans* 3-*O-p*-coumaroylquinic acid and 3-*O*-feruloylquinic acid,
337 respectively. Peak **1** presented a base peak at m/z 191 (quinic acid) **along with** a peak at m/z 163
338 (corresponding to the *p*-coumaroyl acid **moiety**); peak **2** presented the same chromatographic
339 behaviour, leading to the **respective** identification of the *cis* and *trans* isomers of *p*-
340 coumaroylquinic acid. These peaks (**1/2** and **4**) have been previously identified in the foliar
341 parts of *M. oleifera* from South Africa, after being exposed to certain levels of radiation
342 (Ramabulana et al., 2016).

343 The flavonoid was, without any doubt, the most abundant group of phenolic compounds
344 identified in **studied *M. oleifera* samples, with** glycosylated derivatives of quercetin having a
345 superior numerical expression to any other identified flavonoid aglycone. Peaks **10** ($[\text{M}-\text{H}]^-$ at
346 m/z 625), **16** ($[\text{M}-\text{H}]^-$ at m/z 505), and **17/19** ($[\text{M}-\text{H}]^-$ at m/z 549), tentatively identified as
347 quercetin-*O*-dihexoside, quercetin-*O*-acetylhexoside and quercetin-malonylhexoside,
348 respectively, have been previously identified in the leaves of *M. oleifera* from South Africa
349 (Ramabulana et al., 2016), Pakistan (Nouman et al., 2016), and Namibia (**Makita et al., 2016**).

350 Peak **5** presented a pseudomolecular ion $[\text{M}-\text{H}]^-$ at m/z 711, and MS^2 fragments at m/z 667 (loss
351 of 44 u, carboxyl radical), m/z 505 (loss of sinapoylradical), m/z 463 (loss of sinapoyl and acetyl
352 radicals), and m/z 301 (quercetin aglycone), which allowed the tentative identification as
353 quercetin-acetylglucoside-sinapic acid. This peak has not been identified in *M. oleifera*
354 samples, so its tentative identification was performed following the previously described by
355 Medina et al. (2017) in *Passiflora edulis* shell, without numbering the **oxygen atoms** and
356 radicals position since it was not possible to compare the abundance of each fragment. Peak **11**,
357 also a glycosylated derivative of quercetin, presented a pseudomolecular ion $[\text{M}-\text{H}]^-$ at m/z 595,
358 and MS^2 fragments at m/z 463 and m/z 301, corresponding to the loss of a pentosyl and hexosyl
359 moieties, respectively. As peak **5**, peak **11** was not previously identified in ***M. oleifera* samples**,
360 so its tentative identification followed the previously described by Barros et al. (2013) in *Cistus*

361 *ladanifer*. The second major flavonoid group was that of C-glycosylated apigenin derivatives,
362 represented by peaks **7** ([M-H]⁻ at *m/z* 593), **9** ([M-H]⁻ at *m/z* 593) and **12** ([M-H]⁻ at *m/z* 431),
363 tentatively identified as apigenin-6,8-C-diglucoside, apigenin-O-hexoside-C-hexoside, and
364 apigenin-C-hexoside, respectively, following the previously described by Truchado et al.
365 (2011) and Qiao et al. (2011), being previously identified similar compounds in *M. oleifera*
366 leaves (Nouman et al., 2016; Ramabulana et al., 2016). Kaempferol derivatives were also found;
367 peak **8**, tentatively identified as kaempferol-O-malonylhexaside, was previously reported in *M.*
368 *oleifera* leaf samples by Makita et al. (2016), and peak **23**, presenting a pseudomolecular ion
369 [M-H]⁻ at *m/z* 695, was tentatively identified as kaempferol-O-malonyldihexaside, following
370 the previously described by Sánchez-Salcedo et al. (2016) in *Morus* spp. leaves (to the best of
371 the authors' knowledge, this peak as not been described previously in *M. oleifera*). Finally,
372 peak **24**, tentatively identified as isorhamnetin-O-malonylhexaside, was previously described
373 in *M. oleifera* leaves by Ziani et al. (2019).

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

380 The hydroethanolic extracts prepared with flowers from Bissau presented the highest total
381 concentration of phenolic compounds, 14.7±0.1 mg/g of extract, followed by the Quinhamel
382 flower hydroethanolic extract, with 13.8±0.1 mg/g of extract. The seed samples were the only
383 ones presenting flavan-3-ols derivatives, representing the major group of phenolics within this
384 group. Another information that is important to highlight is the fact that the decoction prepared
385 with the Quinhamel fruit sample had no phenolic compounds. Although an aqueous preparation

386 such as decoction can lead to the **thermal degradation of compounds**, the absence of compounds
387 **may be related to the sample itself**, since the hydroethanolic extract of this sample also had the
388 lowest **total concentration of phenolic compounds** (0.765±0.001 mg/g extract) within the
389 corresponding group of samples.

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

397

398 **3.3. Bioactive properties of *M. oleifera* hydroethanolic and aqueous extracts**

399 To evaluate the bioactive properties of the different *M. oleifera* **edible parts**, hydroethanolic,
400 infused and decocted extracts were prepared according to traditional uses and applications.
401 Fruits are traditionally prepared as a culinary vegetable, stewed in curries and soups. In India
402 and Bangladesh, fruits are usually prepared by boiling pods to the desired level of tenderness
403 in a mixture of coconut milk and spices (Lim, 2014). Therefore, only hydroethanolic and
404 decocted extracts were prepared in this study with the fruit samples. On the other hand, seeds
405 and flowers were used to prepare hydroethanolic, infused and decocted extracts. Traditionally,
406 mature seeds are fried and eaten like peanuts in Nigeria and added to sauces for their bitter taste.
407 In Pakistan are used to prepare *M. oleifera* seed tea infusions (Ilyas et al., 2015) and in India
408 seed decoctions (Dhakar et al., 2011). The flowers are cooked and consumed either mixed with
409 other foods or fried in batter, butter or oil. In West Bengal and Bangladesh, these are usually

410 cooked with green peas and potato, while in Africa are eaten as a vegetable, added to sauces or
411 used to make infusions (Lim, 2014).

412

413 *3.3.1. Antioxidant activity*

414 Two *in vitro* cell-based assays were used to measure the antioxidant activity of the
415 hydroethanolic, infused and decocted extracts of the different *M. oleifera* parts (**Table 4**). These
416 assays evaluate the extract ability to inhibit the formation of thiobarbituric acid reactive
417 substances (TBARS) and the oxidative haemolysis (OxHLIA) using porcine brain tissues and
418 erythrocytes as oxidizable biological substrates, *respectively*. As can be observed in **Table 4**,
419 in the TBARS assay, significant differences were found between the three plant parts and
420 between the extraction methods. The hydroethanolic extracts showed the lowest EC₅₀ values,
421 thus translating a greater capacity to inhibit the **TBARS formation** than the aqueous extracts.

422 This result could be justified by the greater efficiency of the hydroethanolic mixture in
423 extracting phenolic compounds and other antioxidants (Padayachee & Baijnath, 2019). In the
424 OxHLIA assay, the sheep erythrocytes were subjected to the haemolytic action of both
425 hydrophilic and lipophilic radicals generated in *in vitro* by the thermal decomposition of the
426 free-radical initiator AAPH and as a consequence of the initial attack, respectively. By
427 observing the data presented in **Table 4**, it can be noticed that infusions prepared with seed and
428 flower samples from Bissau showed the best results, with IC₅₀ values lower than those of the
429 trolox, the water-soluble analog of vitamin E used as a positive control. Interestingly, the
430 hydroethanolic extracts did not show any antihemolytic effect. In a previous study, Pakade,
431 Cukrowska, and Chimuka (2013) compared the antioxidant activity of *M. oleifera* leaves and
432 flowers to that of several vegetables from South Africa, including spinach, cauliflower,
433 broccoli, cabbage, and peas, and reported a total flavonoid content in *M. oleifera* three times

434 higher than that quantified in the others plant foods, thus concluded that *M. oleifera* is a better
435 source of antioxidants.

436

437 *3.3.2. NO-production inhibition activity*

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

453

454 *3.3.3. Cytotoxicity to tumour and non-tumour cells*

455 Considering the described uses of the different parts of *M. oleifera* in traditional medicine, the
456 prepared extracts were also tested for their cytotoxicity for tumour and non-tumour cell lines.
457 The performed sulforhodamine B assay allows to evaluate the effect of the extracts on cell
458 proliferation (Ziani et al., 2019). Therefore, **GI₅₀** values translate the extract concentration

459 providing 50% of cell growth inhibition. As presented in **Table 4**, the hydroethanolic extracts
460 of seed and flower samples originated the lower GI₅₀ values, thus translating a higher activity
461 than the aqueous extracts against HeLa (cervical), HepG2 (hepatocellular), MCF-7 (breast) and
462 NCI-H460 (lung) tumour cells. Among the hydroethanolic extracts, those prepared with seeds
463 were more effective against the HepG2 cell line, regardless of the geographic origin of the
464 samples (with GI₅₀ of 82±5 – 95±2 µg/mL), while those prepared with flowers were more
465 cytotoxic to breast MCF-7 cells (with GI₅₀ of 163±5 – 187±10 µg/mL). For seeds, the
466 decoctions proved to be the least cytotoxic preparations for the tested cell lines (given the higher
467 GI₅₀ values), which is in line with the results obtained with the OxHLIA assay (where they also
468 had the highest IC₅₀ values). The aqueous flower extracts were not cytotoxic at the tested
469 concentrations, nor any of those prepared with the fruits.

470 In previous studies, Jung (2014) found that aqueous *M. oleifera* leaf extracts are able to reduce
471 the proliferation and invasion of cancer cells by inducing apoptosis, inhibiting the tumour cell
472 growth and decreasing the level of internal reactive oxygen species in human lung cancer cells.
473 Al-Asmari and co-workers (2015) evaluated the anticancer properties of *M. oleifera* leaf, bark
474 and seed extracts against breast (MDA-MB-231) and colorectal (HCT-8) cancer cells and
475 obtained remarkable anticancer activities with the leaf and bark extracts, while the seed extract
476 showed less activity. It has also been reported that the flavonoids quercetin and kaempferol
477 present in *M. oleifera* extracts may act as potential chemopreventive agents, being able to
478 reduce the proliferation of human carcinoma through the induction of *in vitro* apoptosis
479 (Padayachee & Baijnath, 2019). In addition, the presence of these and other antioxidants in *M.*
480 *oleifera* allows to reduce oxidative stress and, consequently, help prevent the development of
481 cancer. Among the metabolites with antioxidant activity found in *M. oleifera* are flavonoids,
482 phenolic acids, saponins, tannins, β-carotene, and terpenoids (Singh et al., 2019).

483 **Table 4** also shows that, with the exception of the hydroethanolic seed extracts, no other extract
484 was cytotoxic to the non-tumour PLP2 cells at the tested concentrations. This toxicity of the
485 hydroethanolic seed extracts to porcine liver primary cells may somehow justify the absence of
486 antihemolytic activity in the OxHLIA assay, since the erythrocytes may have been rapidly lysed
487 due to the cytotoxic effect of these hydroalcoholic preparations.

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

503

504 **3.3.4. Antimicrobial activity**

505 The results of the antimicrobial activity of *M. oleifera* extracts are presented in **Table 5**. All the
506 extracts had significant antimicrobial effects against the tested bacteria and fungi. The MIC and
507 MBC values obtained for *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and

508 *Escherichia coli*, as well as for *Enterobacter cloacae* and *Salmonella* Typhimurium, were
509 comparable to those of streptomycin and ampicillin, the antibiotics used as positive controls,
510 thus translating a similar bacteriostatic and bactericidal activity. In general, decoctions were the
511 most effective preparations against the tested bacteria and, in the case of seeds, it is also worth
512 noting the higher activity of the hydroethanolic and infused extracts prepared with seeds from
513 Quinhamel and the decocted extracts made with seed from Bissau. The antimicrobial activity
514 of *M. oleifera* leaf, root, bark and seed extracts against bacteria, yeasts, dermatophytes, and
515 helminths pathogenic to human was previously investigated by Upadhyay, Yadav, Mishra,
516 Sharma, and Purohit (2015), which verified that the seed aqueous extract inhibits the growth of
517 *Pseudomonas aeruginosa* and *S. aureus*. According to previous reports, the antimicrobial
518 activity of *M. oleifera* seed powder is conferred by a short cationic protein (Singh et al., 2019),
519 as well as by saponins, tannins, phenolics, and alkaloids (Padayachee & Baijnath, 2019).
520 The antifungal activity of the tested *M. oleifera* extracts resulted in MIC and MBC values lower
521 or similar to those of the positive controls ketoconazole and bifonazole (**Table 5**). The
522 antifungal activity of aqueous leaf extracts of *M. oleifera* was previously confirmed by
523 Padayachee and Baijnath (2020) against *Penicillium* spp., while the ethanolic extract also
524 inhibited *Candida albicans*, *Penicillium* spp., and *Mucor* spp. The phytochemical screening of
525 this plant part revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids,
526 tannins, and cardiac glycosides, which may act as natural antimicrobials (Padayachee &
527 Baijnath, 2019; Raj et al., 2011).

528

529 **4. Conclusion**

530 The results of the present study highlighted the nutritional **quality** of *M. oleifera* fruits, seeds
531 and flowers **from Bissau and Quinhamel and the bioactive potential of their herbal preparations**.
532 These edible and medicinal **matrices** stood out not only with high nutritional value, but also for

533 their potential to be used in food fortification and in the development of new **functional foods,**
534 **nutraceuticals** and pharmaceutical formulations. *M. oleifera* is a natural resource to be valorised
535 **by underprivileged population facing poverty and malnutrition issues, but also by other**
536 **stockholders, specifically in underdeveloped and developing nations that have an insufficient**
537 **technical resources.**

538

539 **Acknowledgements**

540 The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for
541 financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020), to cE3c
542 (UIDB/00329/2020), and to **the** A. Bancessi PhD grant (SFRH/BD/135356/2017). National
543 funding by FCT, P.I., through the institutional scientific employment program-contract for A.
544 Fernandes, J. Pinela, M.I. Dias, R.C. Calhella, and L. Barros contracts. This work was funded
545 by FEDER-Interreg España-Portugal programme through the project 0377_Iberphenol_6_E
546 and TRANSCoLAB 0612_TRANS_CO_LAB_2_P, and also by the Ministry of Education,
547 Science and Technological Development of Republic of Serbia (451-03-68/2020-14/200007).

548

549 **Declaration of competing interest**

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

552

553 **References**

554 **Adouni, K., Chahdoura, H., Mosbah, H., Santos-Buelga, C., González-Paramás, A.M., Ciudad-**
555 **Mulero, M., Fernandes, A., Calhella, R.C., Morales, P., Flamini, G., Ferreira, I.C.F.R.,**
556 **Achour, L. (2018). Revalorization of wild *Asparagus stipularis* Forssk. as a traditional**
557 **vegetable with nutritional and functional properties. *Food & Function*, 2018, 9, 1578.**

558 Ajibade, T. O., Arowolo, R., & Olayemi, F. O. (2013). Phytochemical screening and toxicity
559 studies on the methanol extract of the seeds of *Moringa oleifera*. *Journal of*
560 *Complementary and Integrative Medicine*, *10*.

561 Al-Asmari, A. K., Albalawi, S. M., Athar, M. T., Khan, A. Q., Al-Shahrani, H., & Islam, M.
562 (2015). *Moringa oleifera* as an anti-cancer agent against breast and colorectal cancer cell
563 lines. *PLoS ONE*, *10*, 1–14.

564 Alhakmani, F., Kumar, S., & Khan, S. A. (2013). Estimation of total phenolic content, *in-vitro*
565 antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pacific*
566 *Journal of Tropical Biomedicine*, *3*, 623–627.

567 AOAC International. (2016). *Official Methods of Analysis of AOAC International* (G. W.
568 Latimer (ed.); 20th ed.). AOAC International.

569 Bancessi, A., Bancessi, Q., Baldé, A., & Catarino, L. (2020). Present and potential uses of
570 *Moringa oleifera* as a multipurpose plant in Guinea-Bissau. *South African Journal of*
571 *Botany*. *129*, 206-208

572 Baptista, A. T. A., Silva, M. O., Gomes, R. G., Bergamasco, R., Vieira, M. F., & Vieira, A. M.
573 S. (2017). Protein fractionation of seeds of *Moringa oleifera* lam and its application in
574 superficial water treatment. *Separation and Purification Technology*, *180*, 114–124.

575 Barros, L., Dueñas, M., Alves, C. T., Silva, S., Henriques, M., Santos-Buelga, C., & Ferreira,
576 I. C. F. R. (2013). Antifungal activity and detailed chemical characterization of *Cistus*
577 *ladanifer* phenolic extracts. *Industrial Crops and Products*, *41*, 41–45.

578 Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P.
579 (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An
580 underexploited and highly disseminated species. *Industrial Crops and Products*, *89*, 45–
581 51.

582 Corrêa, R. C. G., de Souza, A. H. P., Calhelha, R. C., Barros, L., Glamoclija, J., Sokovic, M.,

583 Peralta, R. M., Bracht, A., & Ferreira, I. C. F. R. (2015). Bioactive formulations prepared
584 from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom
585 *Pleurotus ostreatoroseus* Singer. *Food & Function*, 6, 2155–2164.

586 Daba, M. (2016). Miracle Tree: A review on multi-purposes of *Moringa oleifera* and its
587 implication for climate change mitigation. *Journal of Earth Science & Climatic Change*,
588 7, 1–5.

589 Dhakar, R., Pooniya, B., Gupta, M., Maurya, S., Bairwa, N., & Sanwermal. (2011). Moringa:
590 The herbal gold to combat malnutrition. *Chronicles of Young Scientists*, 2, 119.

591 Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive
592 importance and its medicinal application. *Food Science and Human Wellness*, 5, 49–56.

593 Ilyas, M., Arshad, M. U., Saeed, F., & Iqbal, M. (2015). Antioxidant potential and nutritional
594 comparison of moringa leaf and seed powders and their tea infusions. *Journal of Animal
595 and Plant Sciences*, 25, 226–233.

596 Iyda, J. H., Fernandes, Â., Calhelha, R. C., Alves, M. J., Ferreira, F. D., Barros, L., Amaral, J.
597 S., & Ferreira, I. C. F. R. (2019). Nutritional composition and bioactivity of *Umbilicus
598 rupestris* (Salisb.) Dandy: An underexploited edible wild plant. *Food Chemistry*, 295,
599 341–349.

600 Iyda, J. H., Fernandes, Â., Ferreira, F. D., Alves, M. J., Pires, T. C. S. P., Barros, L., Amaral, J.
601 S., & Ferreira, I. C. F. R. (2019). Chemical composition and bioactive properties of the
602 wild edible plant *Raphanus raphanistrum* L. *Food Research International*, 121, 714–722.

603 Jaja-Chimedza, A., Graf, B. L., Simmler, C., Kim, Y., Kuhn, P., Pauli, G. F., & Raskin, I.
604 (2017). Biochemical characterization and anti-inflammatory properties of an
605 isothiocyanate-enriched moringa (*Moringa oleifera*) seed extract. *PLoS ONE*, 12, 1–21.

606 Jung, I. L. (2014). Soluble extract from *Moringa oleifera* leaves with a new anticancer activity.
607 *PLoS ONE*, 9, 1–10.

- 608 Kavitha, C., Ramesh, M., Kumaran, S. S., & Lakshmi, S. A. (2012). Toxicity of *Moringa*
609 *oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish,
610 *Cyprinus carpio*. *Experimental and Toxicologic Pathology*, *64*, 681–687.
- 611 Liang, L., Wang, C., Li, S., Chu, X., & Sun, K. (2019). Nutritional compositions of Indian
612 *Moringa oleifera* seed and antioxidant activity of its polypeptides. *Food Science and*
613 *Nutrition*, *7*, 1754–1760.
- 614 Lim, T. K. (2014). Fruits. In *Edible Medicinal and Non-Medicinal Plants* (1st d., pp. 453–485).
615 Springer, Dordrecht.
- 616 Lockowandt, L., Pinela, J., Roriz, C.L., Pereira, C., Abreu, R.M.V., Calhelha, R.C., Alves, M.J.,
617 Barros, L., Bredol, M., Ferreira, I.C.F.R. (2019). Chemical features and bioactivities of
618 cornflower (*Centaurea cyanus* L.) capitula: The blue flowers and the unexplored non-
619 edible part. *Industrial Crops and Products*, *128*, 496-503.
- 620 Luqman, S., Srivastava, S., Kumar, R., Maurya, A. K., & Chanda, D. (2011). Experimental
621 assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging
622 potential using in vitro and in vivo assays. *Evidence-Based Complementary and*
623 *Alternative Medicine*, *2012*, 1–12.
- 624 Makita, C., Chimuka, L., Steenkamp, P., Cukrowska, E., & Madala, E. (2016). Comparative
625 analyses of flavonoid content in *Moringa oleifera* and *Moringa ovalifolia* with the aid of
626 UHPLC-qTOF-MS fingerprinting. *South African Journal of Botany*, *105*, 116–122.
- 627 Medina, S., Collado-González, J., Ferreres, F., Londoño-Londoño, J., Jiménez-Cartagena, C.,
628 Guy, A., Durand, T., Galano, J. M., & Gil-Izquierdo, A. (2017). Quantification of
629 phytoprostanes – bioactive oxylipins – and phenolic compounds of *Passiflora edulis* Sims
630 shell using UHPLC-QqQ-MS/MS and LC-IT-DAD-MS/MS. *Food Chemistry*, *229*, 1–8.
- 631 Minaiyan, M., Asghari, G., Taheri, D., Saeidi, M., & Nasr-Esfahani, S. (2014). Anti-
632 inflammatory effect of *Moringa oleifera* Lam. seeds on acetic acid-induced acute colitis

633 in rats. *Avicenna Journal of Phytomedicine*, 4, 127–136.

634 Muyonga, J. H., Nansereko, S., Steenkamp, I., Manley, M., & Okoth, J. K. (2016). Traditional
635 african foods and their potential to contribute to health and nutrition: Traditional african
636 foods. In H. U. Shekhar, Z. H. Howlader, & Y. Kabir (Eds.), *Exploring the Nutrition and*
637 *Health Benefits of Functional Foods* (2nd ed., pp. 320–346). University of Dhaka.

638 Nouman, W., Anwar, F., Gull, T., Newton, A., Rosa, E., & Domínguez-Perles, R. (2016).
639 Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from
640 seven cultivars of *Moringa oleifera* Lam. *Industrial Crops and Products*, 83, 166–176.

641 Padayachee, B., & Baijnath, H. (2019). An updated comprehensive review of the medicinal,
642 phytochemical and pharmacological properties of *Moringa oleifera*. *South African Journal*
643 *of Botany*, in press.

644 Pakade, V., Cukrowska, E., & Chimuka, L. (2013). Comparison of antioxidant activity of
645 *Moringa oleifera* and selected vegetables in South Africa. *South African Journal of*
646 *Science*, 109, 1–5.

647 Qiao, X., He, W. N., Xiang, C., Han, J., Wu, L. J., Guo, D. A., & Ye, M. (2011). Qualitative
648 and quantitative analyses of flavonoids in *Spirodela polyrrhiza* by high-performance
649 liquid chromatography coupled with mass spectrometry. *Phytochemical Analysis*, 22(6),
650 475–483.

651 Ramabulana, T., Mavunda, R. D., Steenkamp, P. A., Piater, L. A., Dubery, I. A., & Madala, N.
652 E. (2016). Perturbation of pharmacologically relevant polyphenolic compounds in
653 *Moringa oleifera* against photo-oxidative damages imposed by gamma radiation. *Journal*
654 *of Photochemistry and Photobiology B: Biology*, 156, 79–86.

655 Sánchez-Salcedo, E. M., Tassotti, M., Del Rio, D., Hernández, F., Martínez, J. J., & Mena, P.
656 (2016). (Poly)phenolic fingerprint and chemometric analysis of white (*Morus alba* L.) and
657 black (*Morus nigra* L.) mulberry leaves by using a non-targeted UHPLC-MS approach.

658 *Food Chemistry*, 212, 250–255.

659 Singh, A. K., Rana, H. K., Tshabalala, T., Kumar, R., Gupta, A., Ndhlala, A. R., & Pandey, A.
660 K. (2020). Phytochemical, nutraceutical and pharmacological attributes of a functional
661 crop *Moringa oleifera* Lam: An overview. *South African Journal of Botany*, 129, 209-220.

662 Soković, M., Glamočlija, J., Marin, P. D., Brkić, D., & Griensven, L. J. L. D. van. (2010).
663 Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an
664 *in vitro* model. *Molecules*, 15, 7532–7546.

665 Spréa, R. M., Fernandes, Â., Calhelha, R. C., Pereira, C., Pires, T. C. S. P., Alves, M. J., Canan,
666 C., Barros, L., Amaral, J. S., & Ferreira, I. C. F. R. (2020). Chemical and bioactive
667 characterization of the aromatic plant: *Levisticum officinale* W.D.J. Koch: a
668 comprehensive study. *Food and Function*, 11, 1292–1303.

669 Truchado, P., Vit, P., Ferreres, F., & Tomas-Barberan, F. (2011). Liquid chromatography-
670 tandem mass spectrometry analysis allows the simultaneous characterization of C-glycosyl
671 and O-glycosyl flavonoids in stingless bee honeys. *Journal of Chromatography A*, 1218,
672 7601–7607.

673 Upadhyay, P., Yadav, M. K., Mishra, S., Sharma, P., & Purohit, S. (2015). *Moringa oleifera*:
674 A review of the medical evidence for its nutritional and pharmacological properties.
675 *International Journal of Research in Pharmacy and Science*, 5, 12–16.

676 Zheng, Y., Wu, J., Peng, X., & Zhang, Y. (2019). Field-grown *Moringa oleifera* response to
677 boron fertilization: Yield component, chemical composition of seed-oil and physiology.
678 *Industrial Crops and Products*, 138, 111449.

679 Ziani, B. E. C., Rached, W., Bachari, K., Alves, M. J., Calhelha, R. C., Barros, L., & Ferreira,
680 I. C. F. R. (2019). Detailed chemical composition and functional properties of
681 *Ammodaucus leucotrichus* Cross. & Dur. and *Moringa oleifera* Lamarck. *Journal of*
682 *Functional Foods*, 53, 237–247.

683 **Figure captions**

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

686

687

688 **Supplementary material captions**

689 **Table S1.** Detailed fatty acid composition of *M. oleifera* edible parts.

690 **Fig. S1.** Free sugars profile of *M. oleifera* fruits from Bissau characterized in this study:
691 1- Mobile phase; 2- Fructose; 3- Glucose; 4- Sucrose; 5- Melezitose (PI).

692 **Fig. S2.** Tocopherols profile of *M. oleifera* flowers from Quinhamel characterized in this
693 study: 1- Mobile phase; 2- α -Tocopherol; 3- δ -Tocopherol; 4- Tocol (PI).

Table 1Nutritional value and composition in free sugars, organic acids, main fatty acids, and tocopherols of *M. oleifera* edible parts.

	Seeds		Student's <i>t</i> -test	Flowers		Student's <i>t</i> -test	Fruits		Student's <i>t</i> -test
	Quinhamel	Bissau	<i>p</i> -value	Quinhamel	Bissau	<i>p</i> -value	Quinhamel	Bissau	<i>p</i> -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.

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 (λ_{\max}), and mass spectral data.

Peak	Rt (min)	λ_{\max} (nm)	[M-H] ⁻ (m/z)	MS ² (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-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-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)

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						Fruits			
	Quinhamel			Bissau			Quinhamel			Bissau			Quinhamel		Bissau	
	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Dec	HyEth	Dec
1	nd	nd	nd	nd	nd	nd	4.7±0.1 ^a	1.214±0.01 ^e	1.443±0.003 ^d	3.86±0.02 ^b	1.93±0.03 ^c	0.61±0.01 ^f	nd	nd	0.50±0.01 ^g	0.20±0.01 ^h
2	nd	nd	nd	nd	nd	nd	0.39±0.01*	nd	nd	0.471±0.00*	nd	nd	nd	nd	nd	nd
3	0.178±0.002 ^a	0.035±0.001 ^c	0.10±0.01 ^b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.08±0.01*	0.030±0.003*
5	nd	nd	nd	nd	nd	nd	0.23±0.01 ^a	0.092±0.001 ^c	0.14±0.02 ^b	0.020±0.002 ^d	0.02±0.01 ^d	tr	nd	nd	nd	nd
6	0.44±0.02 ^a	0.081±0.004 ^d	0.07±0.01 ^e	0.10±0.02 ^c	0.28±0.01 ^b	0.29±0.01 ^b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	nd	nd	nd	nd	nd	nd	0.254±0.001 ^c	0.051±0.003 ^f	0.161±0.001 ^d	0.69±0.02 ^a	0.39±0.01 ^b	0.15±0.01 ^c	nd	nd	nd	nd
8	nd	nd	nd	nd	nd	nd	0.262±0.001 ^a	0.098±0.01 ^c	0.15±0.04 ^b	tr	nd	tr	nd	nd	nd	nd
9	0.08±0.01 ^a	0.024±0.004 ^c	0.072±0.002 ^b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	tr	nd	tr	tr	nd	tr	nd	nd	nd	nd
11	nd	nd	nd	nd	nd	nd	0.061±0.001	nd	tr	tr	nd	tr	nd	nd	nd	nd
12	0.05±0.02 ^a	0.008±0.001 ^c	0.010±0.002 ^b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	nd	nd	nd	nd	nd	nd	0.239±0.001 ^c	0.2±0.1 ^d	0.136±0.001 ^f	2.44±0.01 ^a	0.55±0.02 ^b	0.19±0.01 ^d	nd	nd	0.16±0.01 ^e	tr
14	0.041±0.001 ^e	0.050±0.001 ^d	0.003±0.0001 ^f	nd	nd	nd	0.47±0.01 ^a	0.109±0.003 ^c	0.35±0.02 ^b	nd	nd	nd	nd	nd	nd	nd
15	nd	nd	nd	nd	nd	nd	0.678±0.002 ^a	0.100±0.001 ^g	0.159±0.001 ^d	0.55±0.01 ^b	0.326±0.001 ^c	0.10±0.02 ^g	0.126±0.001 ^f	nd	0.20±0.03 ^e	nd
16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.156±0.001	nd	nd	nd
17	nd	nd	nd	nd	nd	nd	2.2±0.1 ^b	nd	nd	3.4±0.1 ^a	0.95±0.01 ^c	0.27±0.01 ^d	nd	nd	nd	nd
18	nd	nd	nd	nd	nd	nd	0.49±0.02 ^d	0.48±0.01 ^d	1.13±0.03 ^b	1.31±0.01 ^a	0.53±0.02 ^c	0.22±0.01 ^e	nd	nd	0.18±0.03 ^f	nd
19	nd	nd	nd	nd	nd	nd	0.75±0.02 ^a	0.184±0.01 ^c	0.71±0.01 ^b	0.10±0.01 ^d	0.073±0.004 ^e	0.020±0.002 ^f	nd	nd	nd	nd
20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.214±0.001	nd
21	nd	nd	nd	nd	nd	nd	0.32±0.01 ^b	0.107±0.001 ^d	0.17±0.01 ^c	0.38±0.01 ^a	nd	nd	0.11±0.01 ^d	nd	nd	nd
22	nd	nd	nd	nd	nd	nd	1.3±0.1 ^a	0.093±0.002 ^d	0.14±0.01 ^c	0.247±0.001 ^b	nd	nd	0.118±0.001 ^c	nd	nd	nd
23	nd	nd	nd	nd	nd	nd	0.83±0.01 ^a	0.19±0.01 ^e	0.39±0.01 ^c	0.76±0.01 ^b	0.254±0.003 ^d	0.113±0.004 ^f	0.11±0.01 ^f	nd	0.12±0.03 ^f	nd
24	nd	nd	nd	nd	nd	nd	0.67±0.02 ^a	0.176±0.001 ^c	0.31±0.01 ^c	0.451±0.004 ^b	0.21±0.01 ^d	0.084±0.003 ^g	0.14±0.01 ^f	nd	0.20±0.03 ^d	nd
TPA	nd	nd	nd	nd	nd	nd	5.1±0.1 ^a	1.214±0.01 ^e	1.443±0.003 ^d	4.33±0.02 ^b	1.929±0.003 ^c	0.61±0.01 ^f	nd	nd	0.579±0.002 ^g	0.231±0.003 ^h
TF3O	0.62±0.02 ^a	0.116±0.001 ^d	0.173±0.001 ^c	0.10±0.02 ^c	0.28±0.01 ^b	0.29±0.01 ^b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TF	0.17±0.01 ^f	0.037±0.001 ^j	0.081±0.002 ^h	nd	nd	nd	8.76±0.03 ^b	1.9±0.1 ^e	3.94±0.04 ^c	10.3±0.1 ^a	3.30±0.04 ^d	1.10±0.02 ^g	0.764±0.001 ⁱ	nd	1.09±0.02 ^g	nd
TPC	0.79±0.02 ^g	0.152±0.002 ^l	0.254±0.003 ^j	0.10±0.02 ^m	0.28±0.01 ⁱ	0.29±0.01 ⁱ	13.8±0.1 ^b	3.1±0.1 ^d	5.4±0.1 ^c	14.7±0.1 ^a	5.23±0.04 ^c	1.71±0.03 ^c	0.764±0.001 ^h	nd	1.66±0.02 ^f	0.231±0.003 ^k

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.

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

		Seeds		Student's <i>t</i> -test	Flowers		Student's <i>t</i> -test	Fruits		Student's <i>t</i> -test
		Quinhamel	Bissau	<i>p</i> -value	Quinhamel	Bissau	<i>p</i> -value	Quinhamel	Bissau	<i>p</i> -value
Antioxidant activity*										
TBARS (IC ₅₀ , 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 ₅₀ , µg/mL)	Hydroethanolic	na	na	-	na	na	-	na	na	-
	Δt = 60 min									
	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
	Hydroethanolic	na	na	-	na	na	-	na	na	-
	Δt = 120 min									
	Infusion	10.1±0.2 b	8.1±0.8 b	0.023	29±1 b	7.8±0.7 b	<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
Anti-inflammatory activity**										
NO-production inhibition (EC ₅₀ , µg/mL)	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 ₅₀ , µg/mL) (cervical carcinoma)	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 ₅₀ , µg/mL) (hepatocellular carcinoma)	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 ₅₀ , µg/mL) (breast carcinoma)	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 ₅₀ , µg/mL) (non-small cell lung cancer)	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 ₅₀ , µg/mL) (porcine liver primary culture)	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. *IC₅₀ 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 IC₅₀ values: 19.6±0.7 µg/mL (OxHLIA, Δt 60 min), 41±1 µg/mL (OxHLIA, Δt 120 min), and 23 µg/mL (TBARS), **EC₅₀ values translate the extract concentration providing 50% of NO-production inhibition. Dexamethasone EC₅₀ value: 16 µg/mL. ***GI₅₀ values correspond to the extract concentration responsible for 50% of cell growth inhibition. Ellipticine GI₅₀ values: 3 µg/mL (PLP2), 1 µg/mL (MCF-7), 1 µg/mL (NCI-H460), 2 µg/mL (HeLa), and 1 µg/mL (HepG2). In each column, for each variable, different letters correspond to significant differences between extracts (*p* < 0.05).

Table 5Antibacterial 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)		MIC/MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	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. funiculosus</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		
	Infusion	0.10/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np	2.50/3.50	0.20/0.25
	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		
	Infusion	0.075/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np	0.20/0.30	0.10/0.20
	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.