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1 **Effects of gamma irradiation and comparison of different extraction methods on**
2 **sesquiterpene lactone yields from the medicinal plant *Thapsia garganica* L. (Apiaceae)**

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4
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23

24 **Abstract**

25 **Ethnopharmacological relevance**

26 *Thapsia garganica* L. roots are used in Algerian traditional medicine for a number of ailments.
27 It is used in a poultice as an antitussive treatment of acute bronchitis and pneumonia, in
28 preparations with milk or oil taken orally to treat common lung diseases, and with the direct
29 application of root sections for the soothing of dental pains.

30 **Aim of the study**

31 The objective of this study was to evaluate the combined effect of microwave assisted
32 extraction and gamma irradiation on sesquiterpene lactones in *T. garganica* extracts

33 **Materials and methods**

34 To evaluate the combined effect of microwave assisted extraction and gamma irradiation on
35 the highly bioactive compounds found in extracts of Algerian *T. garganica*, samples from
36 different locations in Algeria were prepared by extraction from dried leaf and root samples of
37 dried plant material, using different extraction methods. Quantification of the compounds of
38 interest was done using an HPLC. The antioxidant activity extracts was determined using the
39 two free radical scavenging assays: the 2,2-diphenyl-picryl-hydrazyl (DPPH) and the 2,2'-
40 azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS).

41 **Results**

42 It was found that location and extraction method had significant impact on the phytochemical
43 composition of extracts. Gamma irradiation was found to have no effect on the phytochemical
44 composition of the plant extracts or on their antioxidant properties.

46 **Conclusion**

47 The study has shown that microwave assisted extraction is an effective method for
48 investigating chemical compounds in *T. garganica* and the results support the notion that
49 gamma irradiation for sterilization do not alter the chemical composition.

51 *The authors wish to clarify that we cannot recommend the usage of any parts of T.*
52 *garganica, in any form, for any remedy due to its very high toxicity.*

54 **Keywords:** *Thapsia garganica*; gamma irradiation; microwave assisted extraction;
55 thapsigargin; antioxidant

56 **1. Introduction**

57

58 ***1.1. Traditional use in Algeria***

59 *Thapsia garganica* L. (Apiaceae) is a medicinal plant commonly found in Algeria, along the
60 coast, in the plains, in the Saharan Atlas Mountains and in the north of the Saharan desert
61 (Hammiche et al., 2013). It is commonly referred to as: Toufelt in Berber; adhriss by the
62 Kabyle people in the North; thapsie, bounafaa or bou-nafit «that of efficacy» in Arabic; faux
63 fenouil (false fennel) and Thapsia du mont Gargan in French(Hammiche et al., 2013). In
64 English, it is known as the deadly carrot. All parts of the plant are known to be toxic and
65 irritant to the skin, causing blisters, erythema and itching, and the resin of the roots has been
66 found to be particularly toxic (Andersen et al., 2015b). Due to this toxicity *T. garganica* is not
67 allowed in any official pharmaceutical preparation, and we cannot recommend the usage of
68 *T. garganica* roots or fruits, in any form, for any remedy due to its very high toxicity. *T.*
69 *garganica* roots are however used in Algerian traditional medicine for a number of ailments.
70 In Kabylia, the Kabyle people use the root to make a "depurative cure" at the onset of spring
71 (Hammiche et al., 2013). They also use the roots to make a poultice, which is applied to the
72 chest as an antitussive treatment of acute bronchitis and pneumonia. Great care is taken in the
73 preparation and its use is limited; in fact, it is a treatment of last resort when bad weather
74 prevents travel (Hammiche, 2015). If the medical condition is less severe, the oil in which a
75 fresh root is cooked is either rubbed on the chest for its "purgative" properties or ingested in
76 small quantities (Hammiche, 2015). Other traditional uses in Algeria include a preparation
77 with milk or oil taken orally to treat common lung diseases, and the soothing of dental pains
78 with the direct application of root sections (Hammiche et al., 2013).

79 The toxicity of *T. garganica* originates from the presence of thapsigargin (Fig. 1) and other
80 sesquiterpene lactones (Andersen et al., 2015a; Andersen et al., 2017; Drew et al., 2009;
81 Simonsen et al., 2013). Thapsigargin makes up 0.2-1.2% of the dry weight of the plant's roots
82 (Andersen et al., 2015b). The pharmacological activity of thapsigargin is due to its inhibition
83 of the sarco-endoplasmic reticulum Ca^{2+} -ATPase (SERCA) in mammalian cells, which leads
84 to cell apoptosis (Simonsen et al., 2013).

85

86 ***1.2. Antioxidant activity***

87 Both the food and pharmaceutical industries have shown a continuing interest in finding
88 naturally occurring antioxidants for use in the preservation of foods or medicinal products, in
89 order to replace synthetic antioxidants, which are being restricted due to their carcinogenicity

90 and harmful effects on the environment (Prakash et al., 2015). Essential oils from aromatic
91 and medicinal plants, in particular, have been of special interest due to their strong antioxidant
92 activity and antimicrobial constituents in their tissues (Di Venere et al., 2016; Golubović et
93 al., 2014). It has previously been seen that certain Algerian medicinal plants, including *T.*
94 *garganica*, contain strong radical scavengers and can therefore be useful as sources of natural
95 antioxidants for both medicinal and commercial use (Djeridane et al., 2006). However, as
96 many of these plants contain toxic compounds, toxicity issues need to be addressed to ensure
97 the antioxidants are safe to use.

98 ***1.3. Irradiation of medicinal herbs***

99 Medicinal plants are widely used in Algerian folk medicine, especially by the elderly and rural
100 communities with limited access to doctors. However, the plants are subject to deterioration
101 from chemical and microbial processes that occur before reaching the end-user during
102 harvesting, processing, distribution and storage. These processes can alter their efficacy and
103 in some cases their safety, so there is a demand for methods of decontamination and
104 preservation in order to improve consumer safety and therapeutic efficacy. Food irradiation is
105 commonly used to sterilise and to reduce food losses due to spoilage, and it has replaced once
106 commonly used chemical fumigants, like ethylene oxide, and other chemical preservatives
107 that have been reported to be hazardous to human health (Seo et al., 2007). The use of gamma
108 irradiation on food products is approved by the Food and Agriculture Organisation (FAO),
109 the International Atomic Energy Agency (IAEA) and the World Health Organisation
110 (WHO)(Joint, 2009). It has been shown to be a safe, environmentally friendly and energy
111 efficient method to sterilise plant products. It is also a well-established industrial process for
112 the sterilisation of medicinal plants in a number of facilities worldwide and in general do not
113 affect the chemical composition of the leaves and roots (Garg and Gupta, 2016; Seo et al.,
114 2007).

115 ***1.4. Extraction methods***

116 Traditional extraction methods of medicinal plants include decoction or maceration in an
117 organic solvent. These methods however, are highly energy dependent and time consuming.
118 Microwave assisted extraction (MAE) has been found to be a reliable alternative as it requires
119 a lower energy input to result in the same or even higher extraction yields, reduces the use
120 organic solvents, shortens extraction times and improves the reproducibility of results. This
121 extraction method has been used for the analysis of bioactive compounds in a number of
122 medicinal plants (Akloul et al., 2014; Benkaci-Ali et al., 2006; Kennouche et al., 2015).

123 However, care should be taken to choose suitable conditions to avoid the thermal degradation
124 of the analytes of interest. Sample preparation and extraction methods are important to
125 consider when studying medicinal plants, as the methods chosen depend on the target
126 compounds and can affect the phytochemical composition of the final extracts.

127

128 Here we investigate the chemical composition of extracts of *T. garganica* from different
129 regions in Algeria. We evaluate the combined effects of microwave assisted extraction and
130 gamma irradiation on the extraction yield of the bioactive compounds as well as on the
131 antioxidant activity of the extract.

132

133 **2. Materials and Methods**

134 **2.1. Plant material**

135 *Thapsia garganica* L. (Apiaceae) roots and leaves were collected between March and April
136 in 2014 and 2015 during flowering, from two locations in Algeria: Médea (Aïn Boucif) (GPS
137 coordinates N35° 53' 28"/E3° 9' 31") and Béjaia (Kherrata) (GPS coordinates N36° 29'
138 34"/E5° 16' 39"). At each site 50 individuals were sampled. For each individual representative
139 leaf material was taken across the entire plant and roots were dug out. Herbarium vouchers
140 were made for one individual per site. The herbarium vouchers are deposited at the Natural
141 History Museum of Denmark, Herbarium C (C10011584, C10011585; leg. Abir Mohamed
142 Mohamed Ibrahim). The local name, the used plant parts, methods of preparation and
143 administration, and medicinal uses were collected from local inhabitants. Samples were
144 identified Dr. Abdelkrim of the Botanical department at the National School Agronomic,
145 Algiers, Algeria, air-dried and stored at room temperature in the laboratory of chemistry. The
146 collections were made according to Algerian regulations.

147 **2.2. DNA extraction, amplification and sequencing**

148 The taxonomy and species concept of *Thapsia* is not resolved (Weitzel et al., 2014), thus to
149 confirm the identity of the collected samples, total genomic DNA from one sample per site
150 (herbarium accession numbers: C10011584, C10011585) was extracted from 15mg of dried
151 leaf fragments, using the QiagenDNeasy Kit (Qiagen, Copenhagen, Denmark) following the
152 manufacturer's protocol. The nuclear ribosomal internal transcribed spacer (nrITS) region was
153 sequenced as described previously (Weitzel et al., 2014), using primers ITS4 and ITS5.
154 Sequences were edited and assembled using CLC Main Workbench 7 software. BLAST
155 analysis confirmed material from both sites to be *T. garganica* (99% match) as previously

156 identified (Weitzel et al., 2014). The new sequences generated are deposited in GenBank, with
157 the following accession numbers: (submitted to GenBank).

158

159 **2.3. Irradiation**

160 200g of dried root and leaf samples were subjected to the following doses (D) of gamma
161 radiation (values in KGy): D₁:0.1, D₂:0.3, D₃:0.7, D₄:1, D₅:3, D₆:7 and D₇:10; at room
162 temperature in the Centre of Nuclear Research Algiers, Algeria (Centre de Recherche
163 Nucléaire d'Alger, CRNA) with a ⁶⁰Co source. Non-irradiated (D₀:0kGy) samples were used
164 as negative controls. The irradiated samples were kept in the dark and at room temperature
165 (ca 22°C) until analysis.

166

167 **2.4. Extraction and fractionation of plant material of *T. garganica* for HPLC quantification**

168 The individual samples of leaves and roots collected at each site were pooled to make a
169 representative sample of leaves and roots for each area.

170 **Simple extraction (SE):** 1.5 mL of organic solvent (1:1 mixture of 80% MeOH in water and
171 80% Acetone in water) was added to 50 mg of homogenised dried and ground plant material
172 using liquid nitrogen (-196°C) to preserve the samples, then vortexed thoroughly and agitated
173 overnight in a thermomixer (Eppendorf® Thermomixer Compact) at 850 rpm at 25 °C.
174 Samples were then centrifuged for 10 min at 10,000 rpm. 1 mL of the supernatant was
175 evaporated to total dryness in a vacuum concentrator (Scan Speed Maxi Vac Evaporator). 250
176 µL of 80% methanol was then added to re-suspend the extract for HPLC analysis.

177 **Classical maceration (CM):** 40 g samples of dried and ground roots and leaves (irradiated
178 and untreated) of *T. garganica* were submerged in 100 mL of methanol at 40 °C for 10 h under
179 magnetic stirring. After filtration, the methanol extracts were concentrated under reduced
180 pressure to obtain crude extracts and then lyophilized to eliminate all trace of solvent and
181 stored at 4 °C. Before HPLC analysis, 1 mg of each sample was re-suspended in 1 mL 100%
182 methanol.

183 **Microwave assisted extraction (MAE):** 40 g of each dried sample (irradiated and untreated)
184 were ground to powder. Samples were then extracted in 100 mL methanol in a microwave
185 device as previously described (Akloul et al., 2014) for 30 min. The resultant mixture was
186 filtered under vacuum and the filtrate was evaporated to near dryness. The samples were then
187 completely lyophilized and stored at 4 °C. 1 mg of each dry extract was re-suspended in 1 mL
188 100% methanol before HPLC analysis.

189

190 **2.5. Chemical Standards**

191 Standards extracted from the fruits of *T. garganica* were used as references for the
192 identification and quantification of thapsigargin (Tg), nortrilobolide (Nb) and thapsigargin
193 (Tc) (donated by Søren Brøgger Christensen, University of Copenhagen, Denmark). Standard
194 solutions were prepared in triplicate, diluted in 80% methanol (standard dilutions: 5, 25, 50,
195 200, 400, 500, 600, 800, 1000 µg/mL).

196

197 **2.6. HPLC analysis**

198 All samples were filtered in centrifugal filters (Ultrafree® MC GV, 0.22 µm Durapore®
199 PVDF) just before injection into the HPLC. HPLC analysis was performed on an Analytical
200 HPLC-UV Shimadzu Prominence (column oven 30 °C, autosampler 15 °C) and performed on
201 a Kinetix EVO C18 100A column (5 µm, 50 mm× 3 mm; Phenomenex). Acetonitrile (solution
202 A) and milliQ water (solution B) were used as the mobile phase with a flow rate of 0.5
203 mL/min. The gradient program was as follows: 50% A (0–1 min, linear gradient), 100% A
204 (6–9 min, linear gradient), 5% A (14-16 min, linear gradient), 50% A (17-23 min, linear
205 gradient) the flow rate was fixed at 0.5 mL/min. Eluting compounds were detected with UV
206 at 230 nm. Each sample was prepared in triplicate and 10 µl was injected into the HPLC.
207 Calibration curves were generated based on triplicate analysis. To obtain a standard curve for
208 quantification, the calibration graphs were linear in the concentration range 5-1000 µg/mL.
209 The calibration curves for each standard had a correlation coefficient of 0.999.

210

211 **2.7. Antioxidant activity**

212 The antioxidant potential of *T. garganica* root and leaf extracts was determined using the two
213 most widely used free radical scavenging assays: the 2,2-diphenyl-picryl-hydrazyl (DPPH)
214 and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS).
215 All experiments were performed in triplicate for the different concentrations of each plant
216 extract.

217 DPPH assay: The free radical scavenging capability of each extract solution was measured
218 from the bleaching of a purple solution of DPPH as described previously (Şahin et al., 2004).
219 1 mL of methanol solution of 60 µM DPPH was mixed with 26 µL of each of the methanolic
220 extracts of the roots and leaves at different concentrations, 100-1000 mg/mL and 25-200
221 mg/mL respectively. The reaction mixture was carried out in capped glass test tubes. After
222 30min of incubation at room temperature, the absorbance was measured at 517 nm using an

223 optizen Mecasys spectrophotometer. The inhibition percentage of DPPH free radicals (I%)
224 was calculated as follows:

$$225 \quad I\% = (A_0 - A_s / A_0) \times 100$$

226 The DPPH[•] stock solution was freshly prepared before each reaction to reduce the loss of free
227 radical activity during the experiment.

228 ABTS assay: The ABTS method followed (Scalzo et al., 2005) and is based on the capacity
229 of the test samples to scavenge the coloured ABTS radical cation (green ABTS^{•+}), obtained
230 by oxidation with potassium persulphate solution for 12-16 h at 4 °C away from light. The
231 absorption peak of ABTS^{•+} is at 734nm and the addition of antioxidants reduces it to its
232 colourless form. On the day of the assay, the ABTS^{•+} solution was diluted with ethanol until
233 absorbance of 1.00 ± 0.02 at 734 nm. 25 µL of sample extracts were added to 1 mL of the
234 ABTS^{•+} solution. The decrease in absorbance was measured after 7 min of incubation at 734
235 nm. Ethanol was used to set the zero. The radical scavenging activity of the samples tested,
236 expressed as a percentage of the inhibition of ABTS^{•+} (I%), were calculated using the formula:

$$237 \quad I\% = [(A_0 - A_s) / A_0] \times 100$$

238 For both assays, a linear regression was determined and used to calculate the IC₅₀ value. Low
239 IC₅₀ values indicate greater antioxidant activity.

240

241 **3. Results and Discussion**

242 All results presented are averages (±SD) of three repetitions. The treatments were compared
243 by performing a Two-Way factorial ANOVA (Analysis of Variance) on the phytochemical
244 composition of the extracts and a Two-Way ANOVA on the antioxidant activities measured.
245 This was followed by the post-hoc Tukey HSD (honest significant difference) test (95%
246 confidence level) to compare the effect of different conditions on the parameters measured.
247 Values of p<0.05 were accepted as significant. The ANOVA analyses were performed in R,
248 Tukey HSD with the R package agricolae (De Mendiburu, 2014) and the graphs were made
249 using the R package ggplot2 (Wickham, 2009).

250 ***3.1. Effect of the gamma irradiation and extraction technique on the phytochemical*** 251 ***composition in thapsigargins***

252 The results show that across all the three extraction methods, gamma irradiation had no
253 significant effect on the phytochemical composition of the extracts obtained from *T.*
254 *garganica* (Figure 2A, Table 1, Table 2). Thapsigargin is today isolated from plant grown in
255 Ibiza (Spain) and shipped around the world for extraction. We can suggest that in the future

256 the plant material can be safely sterilised by gamma irradiation and thereby add to the
257 conservation of the product during transport.

258 Figure 2B illustrates the effect of the extraction methods on the chemical extracts, which was
259 found to be significantly different from each other in both root extracts (F-value=7.21, P-
260 value=0.001) and leaves extracts (F-value=4.47, P-value=0.01) (Table 2). Simple extraction
261 with liquid nitrogen (SE) of dried *T. garganica* roots and leaves proved to be the least effective
262 method to extract bioactive compounds from small amounts of plant material, with no
263 significant difference between microwave assisted extraction (MAE) and classical maceration
264 (CM). MAE however presents the advantage of being rapid and reproducible as well as
265 requiring less energy than conventional methods like CM (Azwanida, 2015). MAE is known
266 to cause the thermal degradation of certain analytes, but in here, it has been shown to be a
267 suitable method for the extraction of thapsigargin as previously suggested (Benkaci-Ali et
268 al., 2006). The only chemical variations observed between the different extracts were that the
269 Tg content in both the leaves and the roots was higher than Tc and Nt; dried roots of *T.*
270 *garganica* have significantly higher levels of Tg than in the dried leaves (P<0.00, Figure 2B).
271 Nt was the least abundant compound in all the samples. This has already been reported (Smitt
272 et al., 1995), but new localities have been investigated in this study. Clear differences were
273 also seen between the two study regions for the roots only, with the root extracts from the
274 Béjaia region consistently having larger quantities of the three compounds studied (Figure
275 2B). Locality has previously been shown to have an effect on the phytochemical composition
276 of *T. garganica* roots (Drew et al., 2012; Smitt et al., 1995), but the cause(s) of these variations
277 have not yet been identified. We hypothesise that there are biological and environmental
278 factors responsible for these fluctuations. Further investigations are needed to determine the
279 best time to harvest *T. garganica* to optimise Tg extraction, considering that the compound
280 remains extremely expensive at € 187 per mg (Sigma-Aldrich).

281

282 **3.2. Effect of gamma irradiation on the antioxidant properties of *T. garganica***

283 Gamma irradiation was found to have no significant effect on the antioxidant activity of *T.*
284 *garganica* root extracts (Table 3). However, there was significant difference between the
285 scavenging activities of both leaf and root extracts between the Médéa and Béjaia regions, for
286 both the ABTS⁺, leaves (F-value = 4.97, P-value = 0,05), roots (F-value = 8.68, P-value =
287 0.01) and DPPH assays, leaves (F-value = 9.66, P-value = 0.01), roots (F-value = 59.77, P-
288 value = 0.00). It was noted that IC₅₀ values for the DPPH assay were higher than those
289 obtained with the ABTS⁺ assay for the root extracts (Table 3). It has been previously shown

290 that DPPH assays are a rapid and reliable test for the antioxidant capacity of plant extracts,
291 but also an advantageous assay applicable to both hydrophilic and lipophilic environments.
292 The leaf extracts had a much higher scavenging activity than the root extracts with those from
293 the Béjaia region generally higher than the extracts from Médéa. This again shows that there
294 are biological or environmental factors responsible for these fluctuations. As *T. garganica* and
295 other Algerian medicinal plants have been proposed as potential sources of natural
296 antioxidants (Djeridane et al., 2006). This shows that whilst gamma irradiation can be used as
297 a sterilisation method, the locality where a plant is collected can affect its antioxidant
298 potential.

299

300 **4. Conclusion**

301 A difference in the chemical composition of Thapsigargin was observed between different
302 tissues of *T. garganica*. The highest amount of Thapsigargin was found to be in the roots of
303 samples collected in Béjaia. There was a significant effect of locality on the phytochemical
304 composition of the roots but not in the leaves. Locality also affected the antioxidant properties
305 of both the leaf and root extracts.

306 Of the extraction methods used, MAE and CM were equally effective and more efficient than
307 SE to extract bioactive compounds from small amounts of plant material. Gamma irradiation
308 had no significant effect on the phytochemical composition of *T. garganica* as well as the
309 antioxidant activity of the extracts.

310

311 **5. Author contributions**

312 A. Mohamed Mohamed Ibrahim and K. A. Martinez-Swatson established the major part of
313 the results and contributed equally to the manuscript. A. Mohamed Mohamed Ibrahim
314 conducted fieldwork for collects of samples and extraction, prepared samples for HPLC
315 analysis, conducted the antioxidant activity test. K. A. Martinez purified the chemical
316 compounds used as standards and ran the HPLC analysis. F. Cozzi directed and supported the
317 HPLC analysis. F. Benkaci-Ali conceived the project and contributed to the manuscript. F.
318 Zoulikha supervised the writing. H. T. Simonsen initiated, directed and supported the research
319 and writing of the manuscript. All authors edited and approved the final manuscript.

320

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326 irradiation. The Standards were extracted and purified under the direction of Søren Brøgger
327 Christensen and Huizhen Liu at the Department of Drug Design and Pharmacology,
328 University of Copenhagen, Denmark.

329

330

331

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8. Table legends

Table 1: Comparison of the different extraction methods and gamma irradiation doses on the samples from the two study areas Médéa and Béjaia. Values are the mean of three replicates \pm standard deviation (SD) and are expressed as the percentage of compound in the dry weight of the sample (DW%), $P < 0.05$. Tg = Thapsigargin, Tc = Thapsigargin, Nt = Nortrilobolide, CM = Classical maceration, MAE = Microwave assisted extraction, SE = Simple extraction with liquid nitrogen, MR = Médéa roots, ML = Médéa leaves, BR = Béjaia roots, BL = Béjaia leaves. Gamma irradiation doses are given in KGy, significance $P < 0.05$ is compared to the control 0 KGy. Superscript letters within the same row indicate significant ($P < 0.05$) differences in the compound yields between the extraction methods.

Table 2: Factorial two-way analysis of variance on the effect of γ -irradiation (gamma irradiation), extraction method and locality on the chemical variation of the extracts from *T. garganica* with significance displayed as *** $P > 0.00$, ** $P > 0.001$, * $P > 0.01$. df = degrees of freedom, Sum sq = sum of squares, Mean sq = mean square, F_s = F statistic.

Table 3: The effect of gamma irradiation on the antioxidant activity of samples obtained by CM with methanol. Values are represented as the mean IC_{50} (mg/L) value of three replicates \pm standard deviation at 5% significance level. DPPH = 2,2-diphenyl-picryl-hydrazyl assay, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt assay. Gamma irradiation dosages (D) are given in KGy. MR = Médéa roots, ML = Médéa leaves, BR = Béjaia roots, BL = Béjaia leaves.

9. Figure legends

Figure 1: Illustration of structures of the three main sesquiterpene lactones in *Thapsia garganica* L. Thapsigargin (Tg), thapsigargin (Tc), and nortrilobolide (Nt).

Figure 2: A – Stacked bar chart showing the effect of gamma irradiation on Thapsigargin (Tg), thapsigargin (Tc) and nortrilobolide (Nt) levels in the different extracts obtained by the different extraction methods. The dose of irradiation used is shown in kGy with the control (NT = no treatment) and each dosage extraction is represented in a different colors. The amounts presented are relative to the individual samples run. For each irradiation dosage, the extraction methods are represented with shading with the lightest shading being classical maceration (CM), the middle shading is microwave assisted extraction (MAE) and the darkest shading is simple extraction (SE). The size of the bars indicates the percentage of extraction yield, each treatment is responsible for, relative to each compound.

B – Bar plots to show the effect of the extractions methods used on the extraction yield of thapsigargin (Tg), thapsigargin (Tc), and nortrilobolide (Nt) on samples from the two locations. MR = Médéa roots, ML = Médéa leaves, BR = Béjaia roots, BL = Béjaia leaves. In both charts, the amount of the compounds was calculated as the percentage of the compound in the dry weight of the plant material (extraction yield).