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Study of Supercritical CO₂ Extraction from Tunisian *Psidium guava* Leaves. Antioxidant Activity of the Extracts

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ABSTRACT

Guava (*Psidium guajava* L.) is a fruit cultivated in all warm regions of the globe for tasty sweet yellow fruit. In this work the extraction of biologically active constituents of Tunisian guava was performed by supercritical fluid extraction (SFE). The SFE with carbon dioxide (CO₂) and ethanol as co-solvent was explored using two modes of extraction. The SFED (supercritical fluid extraction direct mode) at 20MPa and 333K, and the SFEbS (supercritical fluid extraction by step) mode in six steps using a gradual variation pressure (15- 20 MPa) and temperature (313, 323 to 333K). The obtained results were compared to two traditional extraction methods, the Soxhlet and maceration extractions. All extracts were subjected to the scavenging tests of DPPH and ABTS radicals. The total phenolics and flavonoid contents were measured using standard methods. The obtained results showed that SFE was very rapid compared other used extraction methods. The higher global yield was observed with SFED at 20MPa and 333K (6.92%). However the highest levels of polyphenols (8.19 and 4.30 mg GAE.g⁻¹) and flavonoid (10.8 and 6.55 mg EQ.g⁻¹DW) were observed in the extracts E1 and E2 obtained with SFEbS at 10MPa. The highest radical scavenging effect by DPPH (IC₅₀ = 12 mg mL⁻¹) and ABTS (TEAC= 1.860 mM.mg⁻¹) was observed in maceration extract. Compared to the traditional extraction, SFE method provides low extraction yield, but a best quality of extract in terms of antioxidant compounds. The SFE with progressive variation of pressure and temperature was shown to be the most efficient method based on the extraction selectivity.

Keywords: *Psidium guava*, Total phenolic, Antioxidant activity, DPPH, ABTS, SFE.

INTRODUCTION

Guava (*Psidium guava* L.) is the well-known medicinal plant frequently prescribed in different systems of traditional medicine in Central America and Africa. The use of extracts of leaves, fruits and bark by many people in the world shows the multiple properties of this plant in the prevention and treatment of several diseases. Numerous studies have demonstrated the antioxidant potential of this plant and its properties, hepatoprotective, anti-allergic, antigenotoxic, antiplasmodial, cytotoxic, antitussive, anti-diabetic, anti-inflammatory, antispasmodic and antimicrobial¹. This plant is also used as a hypoglycemic agent and in the treatment of diarrhea, dysentery, infantile viral enteritis and even malaria^{2,3}. These properties have been explained by several studies detailing the specific bioactivity of antioxidants extracted from guava¹. Current research on antioxidants and free radicals have confirmed that fruits and vegetables rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers⁴ and neurodegenerative diseases⁵. The extraction of antioxidants from plants and the quality of the obtained extracts are strongly related to the used extraction

technique. The trend towards green analytical chemistry with new inexpensive and environmentally friendly extraction methods shows the extraction by supercritical fluids (SFE) as a good alternative to conventional solid-liquid extraction techniques. The SFE have a number of advantages in terms of quality of extracted products, cost and environmental pollution. This technique is well suited to the treatment of heat-sensitive substances that cannot be separated by conventional distillation. Indeed the published articles on the use of SFE especially in the pharmaceutical and agri-food sector account 45% of the specific scientific literature⁶. The supercritical CO₂ (SC CO₂) behaves as a lipophilic solvent but, compared to other solvents it has the advantage of an adjustable selectivity or solvent power⁷. It is therefore compatible for the solubilization of lipophilic compounds such as essential oils nevertheless, this low polarity makes it difficult for the extraction of polar compounds, hence the need of polar co-solvents^{8,9}.

Previous study showed that the guava leaves extracts exhibited stronger antioxidant effect than fruit extracts¹⁰. In the present work, the supercritical technology was

applied to Tunisian guava leaves, using SC-CO₂ and SC-CO₂ with co-solvents. Two modes of supercritical fluid extraction were applied in order to optimize the yield and the extracted quality. These SFE methods were compared with traditional techniques as Soxhlet extraction (SE) and maceration extraction (ME).

MATERIALS AND METHODS

Plant material

The leaf samples that are subject of this study were collected from the guava trees growing at Sousse (Tunisia 35°50'Nord 10°38'Est). The leaf samples were washed with tap water and were dried at room temperature (298K) for a period of 25 days in a dry and airy environment. The dried samples were powdered and sieved. The selected particle sizes for extractions were from 0.2 to 0.5 mm. The sample was packaged in plastic bags and stored in a dark dry place until that the extraction was performed.

Extraction methods

Maceration extraction (ME)

The sample of 20 g of leaves powder was immersed in 100 mL of ethanol (Carlo Erba Reagents). The mixture was made in a sterile 125 mL erlenmeyer flask, wrapped in aluminum foil to avoid evaporation and exposure to light and was left for 3 days at room temperature with sporadic agitation. The mixture was filtered three times through Whatman N°2 filter paper and the solvent was evaporated in a vacuum evaporator (Buchi Rotavapor R-205) at 313K and 17.5 MPa. The recovered extracts were stored in domestic freezer until the analysis.

Soxhlet extraction (SE)

Extractions with Soxhlet method were performed using ethanol (EtOH) as solvent. The method consisted of 30 g of leaves powder placed inside a cartridge made by thick filter paper and loaded into the main chamber of the Soxhlet extractor. A 250 mL of solvent were used for extraction, with solvent continuously refluxing over the sample, the total extracting time was 8 h. After the extraction, the solvent was removed by evaporator, and the extraction yield was evaluated. The recovered extracts were stored in domestic freezer until the analyses.

Supercritical fluid extraction (SFE)

The experiments were carried out in the SFE unit shown schematically in Fig. 1. The SFE unit used has been conceived and assembled at the Reactions and Process Engineering Laboratory (LRGP, Nancy, France). The CO₂ used was 99.95% of purity (Messer France). The SFE unit is composed by a stainless steel cell of approximately 125 mL (300 x 23 mm) that supports pressures up to 25 MPa followed up by three cyclonic separators, a cold exchanger, and a hot heat exchanger. The temperature was controlled thermostatically (Huber thermostatic bath) and the pressure was regulated by means of a membrane pump (Dosapro Milton Roy – MilRoyal D), which enabled to reach the extraction pressure, with maximum mass flow rate 3.2 kg/h, which was also connected to a cryostat in order to liquefy the CO₂. An electrical housing allows introducing the desired extraction pressure. The flow rate of the CO₂ was measured by means of a Coriolis force flow

meter (Micro Motion) and, hence, indicated the amount of CO₂ used during the extraction.

Experimental procedure

Based on results of the previous research¹¹, two modes of extraction were carried out: SFED direct mode (20MPa, 333K, 2h), and SFEbS: stepwise extraction in six steps (313K, 10MPa; 323K, 10MPa; 333K, 10MPa; 313K, 20MPa; 323K, 20MPa; 333K, 20MPa), each stage lasts 20 minutes (Fig 2). The extraction procedure consisted of placing 30 g of the sample (ground guava leaves) inside the extraction cell using ethanol as co-solvents. The sample was added between two layers of glass beads ($\Phi = 1.7$ mm) which allow increasing the contact surface between the fluid and the solid matrix. This promotes a uniform distribution, preventing the formation of preferential paths and agglomerations which may decrease the yield of the extraction. The CO₂ leaves the bottle in a gaseous state, at room temperature and the tank pressure of between 5 and 6 MPa, the gaseous CO₂ is then cooled to a temperature of 276 K, using a cold exchanger. The liquid is pumped and then heated to the extraction temperature before being directed to the extractor, which is already at this temperature. At this level, CO₂ is in supercritical state. The unit was pressurized and the sample was kept in contact with SC-CO₂ and co-solvent for 30 min in static mode. After this time, the extractor valve was opened for complete depressurization of the extraction cell, and the intermediate valves between the separators (S1, S2 and S3) were continuously adjusted in order to regulate the pressure and, hence to keep a constant flow rate for each extraction. The temperature in the separators was 293K, and the pressures were respectively 5, 2 and 1MPa in the separators (P1, P2 and P3). The supercritical CO₂ flow rate was between 1.3 and 1.6 g.min⁻¹. Each one of the extractions was performed by triplicate.

After each extraction, the vessels collectors containing extracts were left for 5 min under ambient conditions to assure the complete removal of CO₂. The extracts were concentrated by rotavaporator (Buchi Rotavapor R-205) at 313K and 17.5 MPa. The results from all extracts were evaluated considering the extraction yield, also their quality (antioxidant activity, total phenolic and flavonoids content) performed by the methods described as follows.

Antioxidant contents

The dry residue of the extracts SE, ME and SFE was dissolved in methanol and the obtained solution was used to determine the content of total phenols, flavonoids, and antioxidant activity. Folin-Ciocalteu phenol, Catechin, gallic acid (98%) and all other chemical reagents used were purchased from Sigma Co. (St. Louis, MO, USA).

Total phenol: The total polyphenol content was quantified in the leaves using the Folin–Ciocalteu reagent, according to the modified method previously reported by Singleton and Rossi¹². A 1 mL of diluted extract was transferred to a 25 mL volumetric flask containing 9 mL of ultra pure water, the Folin–Ciocalteu reagent (1mL) was added and mixed. After 3 min, 1 mL of sodium carbonate (15 %) were added. After 30 min of incubation at 313K in the dark, the absorbance was measured at 700 nm using the

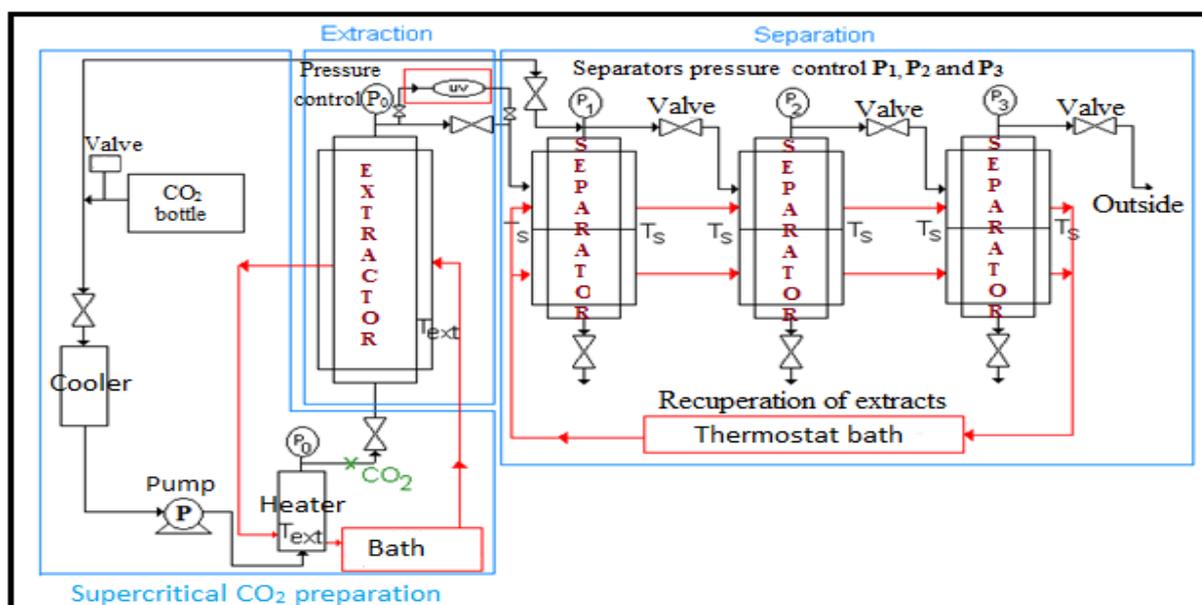


Figure 1: Diagram of dynamic extraction of carbon dioxide

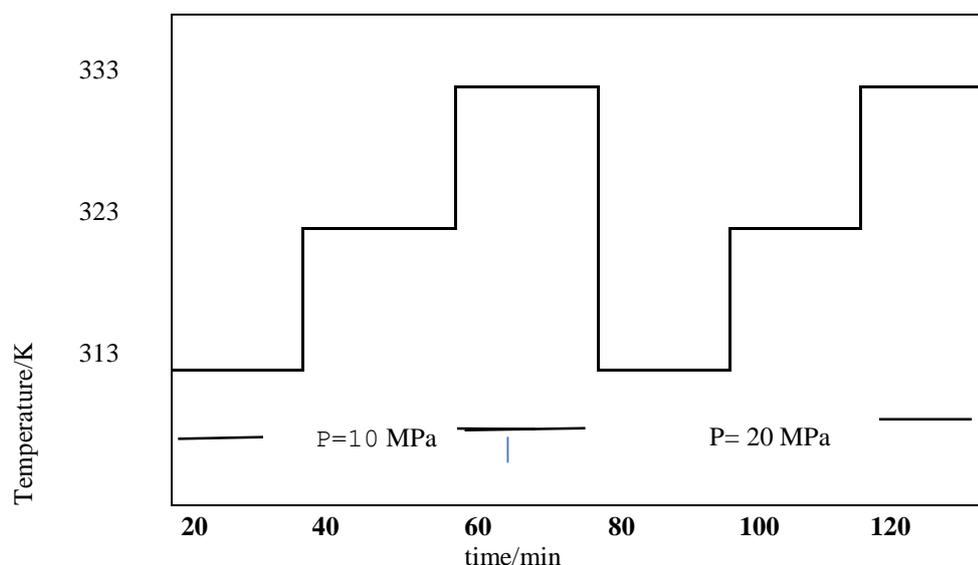


Figure 2: Extraction in six steps (313/10, 323/10, 333/10, 313/20, 323/20, 333/20 K/MPa)

Shimadzu UV-Vis spectrophotometer. The results are expressed as equivalents of gallic acid ($\text{mg GAE} \cdot \text{g}^{-1} \text{FW}$) using a calibration curve of the Gallic acid.

2.3.2 Total Flavonoids: Total flavonoid content was measured according to the modified colorimetric method of Zhishen et al.¹³. Briefly, a 125 μL of leaves methanol extract was added to a 75 μL of NaNO_2 (5%). The mixture was incubated for 6 minutes. A 150 μL of $\text{AlCl}_3 \cdot 6 \text{H}_2\text{O}$ (10%) freshly prepared are added, after 5 minutes of incubation, 500 μL of NaOH (solution 1M) added to the mixture, the final volume was adjusted to 2500 μL with distilled water. The absorbance was measured at 510 nm in a spectrophotometer (Shimadzu UV-Vis spectrophotometer). The blank was prepared using the

same procedure with ultra pure water without extract, and each measure was made in triplicate. A series of methanolic dilutions of quercetin were prepared and assayed; flavonoid amounts in extract were expressed in mg quercetin equivalent flavonoid / g dry matter ($\text{mg QE} \cdot \text{g}^{-1}$ of DM).

Antioxidant activity

DPPH scavenging method

The DPPH radical method was based on the procedure proposed by Burda and Oleszek¹⁴ using 96-well microplates in a spectrofluorimeter (Flx-Genius, SAFAS-France) coupled with an automatic injector comprising two 1 mL syringes (SAFAS-France) and a water-bath (Fisher-France). DPPH "2,2-diphenyl-1-picryl-hydrazyl" (Ref. D

Table 1: Methods of extraction

Supercritical fluid extraction (CO ₂ /Ethanol)						
Extract (SFEbS)	T (°C)	Extraction time (h)	P (MPa)	Pressure separateur	Yield (%)	Yield global (%)
E1	40-50-60	1	10	5	1,55	6,1
E2				2	1,75	
E3				5	2,5	
E3'				2	0,30	
Extract (SFED)						
E4	60	2	20	5	6,95	7,05
E4'				2	0,10	
Other extraction methods (Ethanol)						
Extract	T (°C)	Extraction time (h)			Yield (%)	
S1	69	8			13	
M1	25	72			11,5	

E1, E3, E4: extract recovered separator S1

E2, E3', E4': extracts recovered separator S2

M1 : extract by maceration; S1: extract by soxhlet

SFED: Supercritical fluid extraction direct mode

SFEbS: Supercritical fluid extraction mode by step.

-9132) was purchased from Sigma-Aldrich and ethanol from Carlo Erba. Briefly, it was used 220 μL DPPH solution 46.7mg.L⁻¹ in ethanol, with absorbance measured at 517 nm (A₀). Then, 80 μL of extract in ethanol at different concentrations (0.125 to 1 mg. L⁻¹) were added and the absorbance was measured after 1 h (A_f). The percentage of inhibition (%I) of free radical DPPH by extract sample was calculated using the formula given below: $\%I = ((A_0 - A_f) / A_0) \times 100$

The results are expressed on the bases of IC₅₀ values, defined as the concentration of the sample or the reference compound to decrease the absorbance at 515 nm (or concentration) of DPPH solution to half of its initial value.

ABTS scavenging method

Antioxidant activity of leaves extract was analyzed by investigating their ability to scavenge the ABTS•+ free radical using the modified method previously reported by Ozgen et al.¹⁵. The stock solutions included 7 mM ABTS solution and 4.9 potassium persulfate solutions. Trolox "6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid" from Sigma-Aldrich (Ref. 56510). The working solution was prepared by mixing the two stock solutions in equal proportions and allowing them to react for 16 hours before use in order to produce ABTS radical (ABTS•+). This solution was stored in a dark place at room temperature. Before use, the solution was diluted with ethanol to obtain absorbance between 700 nm and 800 nm. This solution was mixed with sample (5 to 40 $\mu\text{g.mL}^{-1}$). A control containing methanol and ABTS•+ solution was also realized. The absorbance was read at 734 nm after 30 min incubation at 303 K. As unpaired electrons are sequestered by antioxidants in the sample the test solution turns colorless and the absorbance at 734 nm is reduced. All measurement was performed in triplicate. The percentage inhibition was calculated against a control and compared to a Trolox standard curve. The results are expressed in

terms of TEAC (Trolox equivalent antioxidant capacity). This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract¹⁶.

Statistical analysis

All assays were performed in triplicate for each extracting condition. An analysis of variances (ANOVA) for each experiment (yield and quality evaluation) was carried out. The results are reported as standard deviation \pm SD (standard deviation) obtained from the three measurements.

RESULTS AND DISCUSSION

Extraction yield

The obtained yields by SFED, SFEbS, SE and ME are presented in Table 1. The SFE Results were conducted to evaluate the pressure and temperature effect. They showed that the yield of the extract obtained by SFED was slightly higher (7.05 %) than that obtained by SFEbS (6.1%). This yield is higher than that given by Moura et al. (2.9%) for SFE extract of guava Brazil leaves at 328K and 30 MPa¹⁷. These results showed that the yield increases directly with pressure and temperature. With SFED, the pressure and temperature are high for the entire duration of the extraction (2h). The density of the supercritical CO₂ under pressure is increased which increases of the solvent power, however the extraction is less selective, and a large amount of non-phenolic compounds can be driven during extraction. On the other hand Marongiu et al. indicated that increasing the CO₂ density permits the extraction of compounds of high molecular weight¹⁸. By against with SFEbS the changes of temperature and pressure make the extraction more selective that can produce various fractions more or less enriched in phenolic compounds according to the temperature and pressure of the extraction step. The (SE) and (ME) lead to higher yields (13 % for

Table 2: Quality results in terms of antioxidant activity (by DPPH and ABTS methods) and TP, TF, for SFE, SE and ME.

Extract	TP mg GAE.g ⁻¹ DW	TF mg EQ.g ⁻¹ DW	TEAC(ABTS) mM.mg ⁻¹	IC50(DPPH) µg mL ⁻¹
E1	4.30 ± 0.60	6.55 ± 0.10	0.140	155.97
E2	8.19 ± 0.85	10.83 ± 1.05	0.210	105.98
E3	0.66 ± 0.02	0.30 ± 0.01	0.160	139.06
E4	3.29 ± 0.50	0.97 ± 0.01	0.170	127.71
M1	4.07 ± 0.35	4.50 ± 0.45	1.860	12
S1	1.92 ± 0.02	1.35 ± 0.08	1.141	19.63

TP: Polyphenols (mg GAE.g⁻¹ DW); TF: Flavonoids(mg EQ.g⁻¹)

SE; 11.5% for ME). The yield of ME is similar to the value given by Mailoa et al.¹⁹ for guava Indonesia leaves (11.37%).

Antioxidants contents

Total phenolic contents (TPC) expressed in mg gallic acid equivalent.g⁻¹ DW are present in table 2. The three extraction methods differed mainly in the total percentage of phenolics extracted. SFE extracts gives the highest content. Previous studies has shown that the SFE extraction is not the best method to extract polyphenols¹⁹ but the using a polar co-solvent with the CO₂ can significantly improve the extraction yields.

Total phenolic contents obtained with SFE extracts (E1+E2+E3) are three times greater than that obtained with maceration extracts (4.07) and six times greater than that obtained with Soxhlet extracts (1.92). The supercritical fluid has high diffusivity, low viscosity and low surface tension, which gives it attractive characteristics as an extraction solvent of components from solid matrix²¹.

The extracts obtained by SFE at 10MPa contain the highest levels of polyphenols (4.3 and 8.19 mg GAE.g⁻¹ respectively for E1 and E2). By comparing the obtained results for TPC with those reported in the literature, the tested extracts in this study have a lower TPC compared with those reported for aqueous extracts obtained from Taiwan guava leaves¹⁰ (414 to 483 mg GA/ g) and for ethanolic Soxhlet extracts from Brazil guava leaves²² (12.72 to 44.02 mg GAE/g). The flavonoid contents (TF) of the extracts expressed in quercetin equivalent ranged from 0.30 ± 0.01 to 10.83 ± 1.05 mg EQ.g⁻¹DW (Table 2). The results showed the presence of flavonoid in all the extracts t with predominance of SFEbS extracts (E1 and E2). At high pressure and temperature (333K /20MPa) the levels of flavonoids are low (0.97 mg EQ.g⁻¹DW in E4). Presumably, the high temperature leads to chemical changes (most probably oxidation) of the phenolic compounds (including flavonoids) and to lower amounts of these substances detected in the extracts. The SFE of phenolics compounds and flavonoids from guava leaves, with CO₂ and ethanol as co-solvent and with progressive variation of pressure and temperature, was found to be a fast and effective extraction method, with high selectivity compared to SFED and to maceration and Soxhlet extraction.

Antioxidant activity

Radical-scavenging activity on DPPH

Recent studies have shown that there is no universal method to evaluate antioxidant activity quantitatively and accurately²³. Therefore, the antioxidant activity of plant species is generally evaluated using several methods. In this study, two free radicals were used to assess the potential free radical-scavenging activities of guava extracts, namely DPPH• and ABTS•+ radical.

The traditional extraction methods (SE and ME) exhibit a relatively high absorption efficiency of antioxidants contained in guava leaves. The extracts obtained by these methods show the highest antioxidant activity IC₅₀ (ME) = 12.00 and IC₅₀ (SE) = 19.63 µg mL⁻¹. The SFE extracts show that the composition of antioxidants (polyphenols and flavonoids) and the antioxidant activity not only depend on the operating conditions (temperature and pressure) but on the pressure of the recovery separator. The extracts E1 and E2 recovered respectively on separators S1 (5 MPa) and S2 (2 MPa) after one hour of extraction at 10PMPa showed respectively 1.75% and 1.55% yields with some differences in terms of antioxidant activity. The extract E2 has better quality compared to E1 (IC₅₀ (E2) = 105.98; IC₅₀ (E1) = 155.97 mg.L⁻¹) and an increased amount of polyphenols (TP = 8.19; 4.30 mg GAE.g⁻¹ DW). The yields extracts of E3 (SFEbS) and E4 (SFED) obtained at 20MPa are respectively 2.5% and 6.95% and have similar antioxidant activity (IC₅₀ (E4) = 127.71 and IC₅₀ (E3) = 139.06) higher than E1 extract. This latter is richer in phenolic compounds than E3 and E4 (4.30; 0.657; 3.29 mg GAE.g⁻¹ DW). This suggests that these extracts are rich in antioxidant compounds more active and less volatile than the polyphenols such as saponins di- or tri-terpenoids abundant in the guava leaves¹⁹. The extracts obtained in this study have a higher antioxidant activity compared to those reported by Silva et al.²⁴ obtained for two extracts SC CO₂ extracted from Brasilia guava leaves (IC₅₀ = 217.1 mg L⁻¹ at 15MPa / 323K and 197.0 mg L⁻¹ at 15MPa / 333K).

3.3.2 Radical-scavenging activity on ABTS

The ABTS assay was used to confirm the DPPH results since they have a similar antioxidant mechanism and the extracts used in both tests were ethanol-soluble. The scavenging activity of the extracts on free radical ABTS was compared with a standard amount of Trolox. The results calculated as Trolox equivalent antioxidant capacity (TEAC), are shown in Table 2.

As in the case of DPPH tests all extracts presented the free radical-scavenging property but with different degrees.

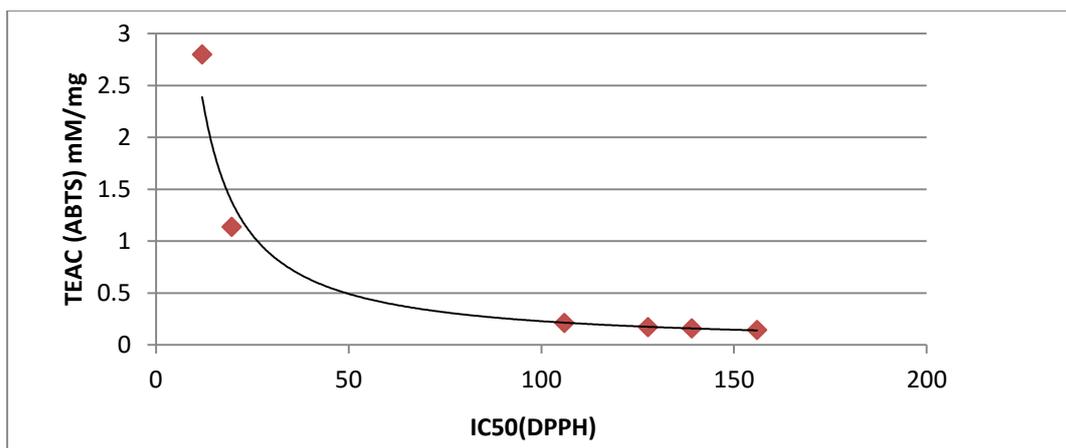


Figure 3: Correlation of antioxidant activity of leaf extract from DPPH (IC50) and ABTS (TEAC) assay

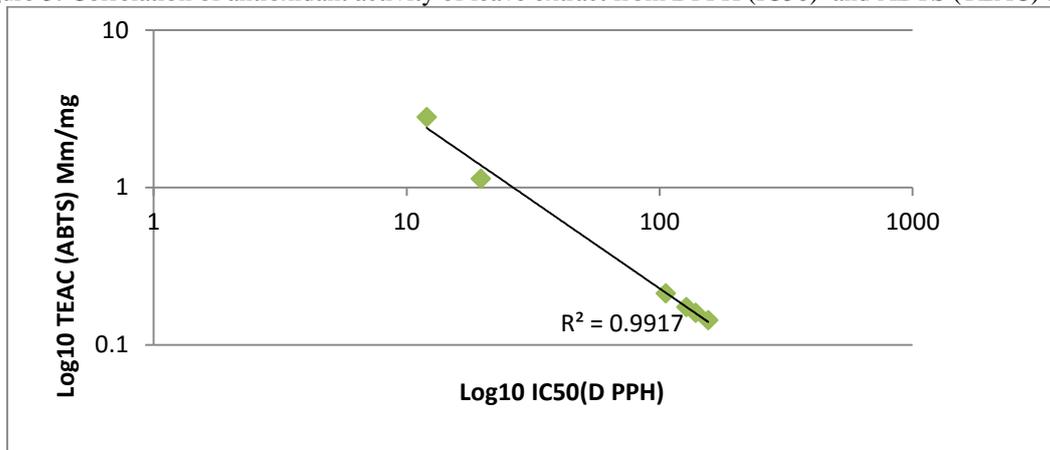


Figure 4: The linear relationship of logarithmic values of IC50 and TEAC

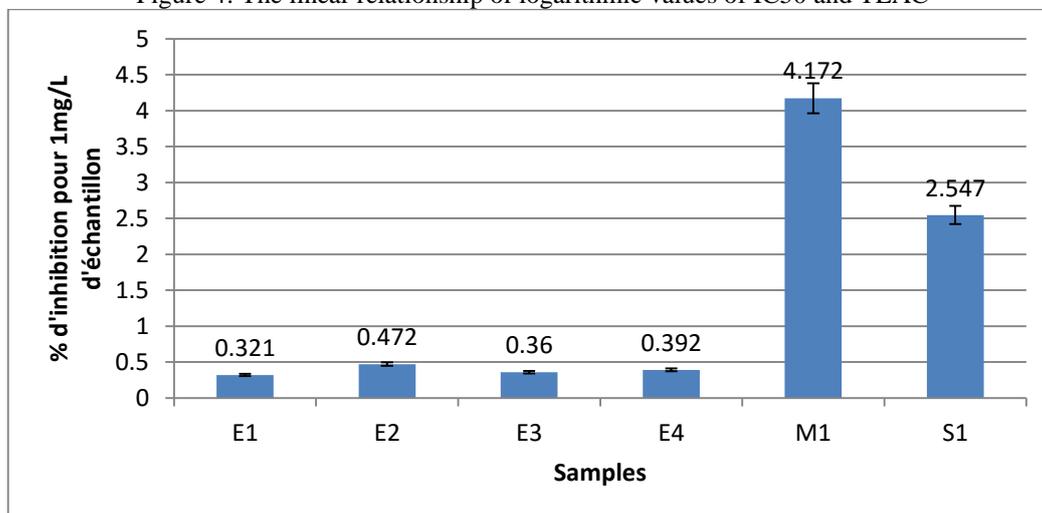


Figure 5: Percent inhibition of DPPH by extracts of leaves

The TEAC values ranged from 0.140 to 1.860 mM/mg. The extract of ME exhibited the highest TEAC value (1.860), followed by SE extract with TEAC value of (1,141). The lowest TEAC value, which indicated the weakest antioxidant activity, was obtained with E1 extract (0.140). These results were in agreement with DPPH assay. As shown in figure 3 the relationship between TEAC and IC50 values was non-linear. However, the plot of logarithmic values of IC50 against TEAC gave good

linearity with $R^2 = 0.9917$ (Fig. 4) indicating good correlation in the radical-scavenging activities. According to these results, it could be considered that tunisian Guava contained strong antioxidative agents, and maceration extraction gives the extract with the highest potential. Tableau 1: Yield obtained in different extraction processes. *Impact of extraction methods on antioxidants extracted from guava*

For all evaluated samples, SFE showed to be the best technique to obtain higher number of phenolic compounds in guava leaves with time consuming steps and without solvent residues. We noted that the traditional processes had reduced ability to extract functional compounds mainly the SE. This is due to the fact that high temperature used in the Soxhlet method can degrade thermolabile compounds. In most cases, the conventional methods are not selective and need various stages of fractionation to obtain the desired compound. Consequently, lost of compounds can occur along these fractionation steps. However in our case, the (ME) presented good results, combining product quality (antioxidant activity) and extraction yield (Fig 5)

CONCLUSION

The global yield and antioxidant activity of tunisian *guava* leaves extracts obtained by SFE with various experimental conditions involving two conventional extraction processes were determined in this study. The highest global yield was obtained with the Soxhlet extraction process but this process leads to a large amount of non-phenolic and non-flavonoid material. The extract by maceration presented the best activity antioxidant and good content of antioxidant. The SFE was a rapid process that leads to a lowest global yield than conventional processes but provides a great number of phenolics compounds. In SFE the higher global yield was observed at high temperature and pressure (in SFED mode). The process with progressive variation in temperature and pressure (SFEbS mode) provides the best results in term of antioxidant activity and antioxidant contents. It provides various fractions with different chemical composition and antioxidant activity. Compared to maceration and Soxhlet extraction, SFE provides high quality of extract, that requiring short time frames and less labour. The SFE (in SFEbS mode) was shown to be the most efficient method based on the extraction time and selectivity. This study showed that, with a progressive variation of the temperature and pressure, the SFE is even more selective and can produce various extracts of different concentrations of phenolic and non-phenolic, volatile and nonvolatile antioxidant compounds.

REFERENCES

- Gutierrez RMP, Mitchell S. et Solis, RV. Goyavier : un examen de ses utilisations traditionnelles, phytochimie et de la pharmacologie. J. Ethnopharmacol.2008;117: 1-27
- Oh W.K., Lee C.H., Lee M.S., Bae E.Y., Sohn C.B., Oh H., Kim B.Y., Ahn J.S. Antidiabetic effects of extracts from *Psidium guajava*. Journal of Ethnopharmacology 2005; 96: 411–415.
- Ojewole, J.A. Hypoglycemic and hypotensive effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract. Methods and Findings in Experimental and Clinical Pharmacology. 2005; 27: 689–695.
- Kris-Etherton P. M., Hecker K. D., Bonanome A., Coval, S. M., Binkoski A. E., Hilpert K. F. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine. 2002; 113: 71S - 88S.
- Di Matteo V., Esposito E. Biochemical and therapeutic effects of the antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Current Drug Target CNS Neurological Disorder. 2003; 2: 95 - 107.
- Capuzzo A., Massimo E. Maffei, Andrea Occhipinti. Supercritical Fluid Extraction of Plant Flavors and Fragrances. Molecules 2013; 18: 7194 -7238
- Reverchon E. Supercritical fluid extraction and fractionation of essential oils and related products. J. Supercrit. Fluids 1997; 10: 1-37.
- Herrero M., Castro-Puyana M., Mendiola J.A., Ibanez E. Compressed fluids for the extraction of bioactive compounds. Trends Anal. Chem. 2013; 43: 67–83.
- Diaz-Reinoso B.; Moure A.; Dominguez H.; Parajo J.C. Supercritical CO₂ extraction and purification of compounds with antioxidant activity. J. Agric. Food Chem. 2006; 54: 2441–2469.
- Hui-Yin Chen, Gow-Chin Yen. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chemistry. 2007; 101(2): 686-694.
- Castro-Vargas H I., Rodríguez-Varela L.I., Ferreira S. R.S., Parada-Alfonso F. Extraction of phenolic fraction from guava seeds (*Psidium guajava* L.) using supercritical carbon dioxide and co-solvents. J. of Supercritical Fluids. 2010; 51: 319-324
- Singleton V. L., Rossi J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16 (3): 144 -158.
- Zhishen J., Mengcheng T., Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 1999; 64(4): 555-59.
- Burda S., Oleszek W. Antioxidant and Antiradical Activities of Flavonoids. J. Agric. Food Chem. 2001; 49: 2774-2779
- Ozgen M., Reese R. N., Tulio A. Z., Scheerens J. C., Miller A. R. Modified 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) methods. Journal of Agricultural and Food Chemistry 2006; 54(4): 1151-1157.
- Antolovich M., Prenzler P. D., Patsalides E., McDonald S., Robards K. Methods for testing antioxidant activity. Analyst. 2002; 127: 183-198.
- Moura P.M., Prado G.H.C., Meireles M.A.A., Pereira C.G. Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data. J. of Supercritical Fluids. 2012; 62: 116 – 122.
- Marongiu B., Piras A., Porcedda S., Casu, R. and Pierucci P. Chemical composition of the oil and

- supercritical CO₂ extract of *Schinus molle* L. Flavour and Fragrance J. 2004; 19: 554-558.
19. Mailoa M.N., Mahendradatta M., Laga A., Djide N., Tannin Extract Of Guava Leaves (*Psidium Guajava* L) Variation With Concentration Organic Solvents International Journal of Scientific & Technology Research. 2013; 2: 106-110.
20. Kőszegi K., Vatai G., Békássy-Molnár E. Comparison the Soxhlet and Supercritical Fluid Extraction of Nettle Root (*Urtica dioica* L.). Periodica Polytechnica Chemical Engineering. Online 2015; paper 7582. DOI: 10.3311
21. Taylor L.T., Supercritical Fluid Extraction. John Wiley & Sons Inc., New York 1996.
22. Sena S. R. C., Dantas T.R.F., Pereira C.G. Evaluation of the antioxidant activity of extracts from *Psidium guajava* L. and *Anacardium occidentale* L. leaves obtained by different extraction methods. Journal of Food, Agriculture & Environment. 2014; 12: 93-97.