IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
April 2020 Vol.:15, Issue:2

Effects of Operating Parameters on the Recovery of Antioxidant Phenolic Compounds of the Roots of *Ximenia americana* by Microwave Assisted Extraction

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Kenmogne Sidonie Beatrice*[1], Tsatsop Tsague Roli Karole [2], Nouga Bissoue Achille[3], Momeni Jean[4], Kamdem waffo Alain[1], Ngassoum Martin Benoit[2]

[1]Department of Chemistry, Faculty of Sciences, University of Douala, P.O. Box. 24157 Douala, Cameroon

[2] Laboratory of Applied Chemistry, Department of Applied Chemistry, P.O.Box. 455. National Advanced School of Agro-Industrial Sciences, Ngaoundere, Cameroon

[3] Advanced Teacher's Training School for Technical Education, University of Douala, Douala 1872, Cameroon

[4]Department of Chemistry, Faculty of Science, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon

Submission: 22 March 2020
Accepted: 29 March 2020
Published: 30 April 2020

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: *Ximenia americana*; roots; total polyphenolic; total flavonoid; antioxidant activity; microwave assisted extraction

ABSTRACT

Microwave-assisted extraction (MAE) has emerged as an efficient extraction technique for various kinds of biological samples due to its low usage of extraction solvents and shorter extraction time. In this study, the extracts were characterized regarding the contents of total polyphenols and flavonoids of *Ximenia americana* roots. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. Some parameters influencing its extraction efficiency was study and the optimum conditions to obtain a high content of total polyphenols and flavonoids was achieved using 80%:v/v binary methanol-water solvent to extract *Ximenia americana* powdered roots with particle size of 0.6mm and plant solvent to solid ratio of 20 mL/g. Four extraction methods were also tested, the highest total polyphenolic and total flavonoid content were detected in Microwave extract with maximal values of 4992.07 ± 11.25 µg GAE/g and 161.71±10.18 QE/g, followed by Soxhlet extract (4339.31 ± 10.35 µg GAE/gand 141.86±1.02 QE/g), maceration extract (3136.00 ± 9.23 µg GAE/gand 103.41±1.71QE/g) and finally by ultrasound extract (1114.12 ± 5.65 µg GAE/g and 15.31±1.57QE/g). Besides that, the extraction time of MAE was the shortest which is only 80 seconds. However, the best value of $IC_{50,}$ knowing as the concentration of the phenolic compounds that caused 50% inhibition of DPPH was similar and important (IC_{50} = 49.24 ± 3.14 µg/mL) for ME and (IC_{50}= 55.87 ± 2.21 µg / mL) for MAE. A good correlation was observed between this antioxidant activity and the total flavonoids (R^2 =79.33%, P<0.05). Our study emphasize on MAE usage as a promising method for the preparation of bioactive phenolic extracts that could be used in small scale industrial application in Cameroon.

INTRODUCTION

Ximenia americana is a plant which grows widely in the tropical and temperate regions of the world. It is extensively used among the Hausa/Fulani communities in the Northern parts of Cameroon and Nigeria as herbal remedies in treating malaria, leproutic ulcers and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). Arbonnier (2004) reported the medicinal uses of *X. americana* to include treatment of fever, stiffness, onchocerciasis, sore throat, asthma and headaches. The roots are used for treating abdominal pains, dysentery, inflamed joints and mouth ulcers (Ake and Guinko, 1991; Mapongmetsem *et al*., 2008). Phytochemical screening of the leaves revealed the presence of saponins, cyanogenic glycosides, flavonoids and tannins (Ogunleye and Ibitoye, 2003; Mapongmetsem *et al*., 2012). However, this valuation requires a critical step namely the extraction of active biomolecules. Also, the search for efficient techniques is topical. Conventional extraction techniques, such as liquid-liquid extraction (LLE), solid-liquid extraction (SLE) and Soxhlet extraction, are still used extensively in present extraction and analytical procedures (You *et al*. 2012; De Sousa *et al*. 2012; Zhou *et al*. 2012). However, these traditional extraction methods are often time-consuming with low extraction efficiencies, and also require large volumes of hazardous organic solvents. For the past two decades, there has been a steady progress in extraction technology, resulting in significant improvements to existing methods, as well as the development of new sample preparation techniques such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), ultrasound assisted extraction (UAE), pressurized hot water extraction (PHWE) and microwave-assisted extraction (MAE) (Tadeo *et al*., 2010; Teo *et al*., 2010; Mustafa and Turner 2011; Zhang *et al*., 2012; Rolly *et al*.,2016). Kenmogne *et al*., (2014) have been reported that the microwave irradiation is an efficient tool to improve the performance of the bioactives compound extraction from *Ximenia americana* roots. However, studies involving the MAE of bioactives compounds from *Ximenia americana* roots can still be hardly found, especially to understand the effects of operating parameters such as microwave irradiation time, microwave irradiation power, type of solvent, solvent to solid ratio and polarity of solvent.

MATERIALS AND METHODS

Plant Materials and Chemicals

Raw material and chemicals

The roots of *Ximenia americana* used in this study were collected at Ngaoundere (Cameroon, central Africa) forest. The identities of plants were verified by comparing the collected specimens with those in the Ngaoundere University by Prof. Mapongmetsem p.m. The collected roots were sun dried for seven days, grounded in an electric grinder (IKA-Universal muhle M20, Germany) and sieved with mechanical shaker analysensieb, Model NFX 11-501, Germany, through a standard set of stainless steel sieves. Three particles fraction were collected and used in the experiment. Chemicals were from Sigma-Aldrich and were used as received. Microwave-assisted extraction experiments were performed with a house domestic microwave (2450 MHz, Toshiba and Tokyo, Japan) with a variable power up to 1000 watts and time.

Microwave assisted extraction

Once the suitable solvent type was determined, it will be employed in this. Samples of 2.5g of crushed dry roots from *Ximenia americana* with three particles sizes (0.2mm,0.6mm and 1mm leaves) were mixed with different solvents: distilled water, water/methanol mixtures (20/80, 40/60, 60/40, 80/20 and 90/10 v/v) and methanol with different sample solvent volume to weight ratios: 5/1,10/1, 15/1, 20/1, 25/1 and 30/1 (v/w) then were suspended in a screw-cap vials. The suspensions were irradiated for 160 sec in a microwave oven (ER- 696 ETE, 2450MHz, and Toshiba, Japan). The suspensions were not allowed to boil; therefore, after each 20 sec, they were cooled to room temperature of 23^{o}C for 3min. The irradiation step was repeated up to six times of 20,40,60,80,100,120,140 and 160 sec and irradiation power of 200w, 400w, 800w and 1000 w were studied. These parameters are determined based on literature for MAE of polyphenols from plant and the limitation of the equipment. After each irradiation, the samples were filtrated using Wattman paper and evaporated under vacuum. The weight of dry residues was regarded as the content of bioactive components in the root of the plant.

Ultrasonic assisted extraction (UAE)

Extraction was done in an open ultrasonic bath (BRASON, B-220, Ultrasonic cleaner). The best solvent, optimum particle size, optimum solid-to-solvent ratio and optimum solvent ratio previously determined were employed in the study. The mixture was boiled for two hours. The solution has been filtered, and the filtrate obtained was concentrated in a rotary evaporator.

Soxhlet extraction (SE)

Extraction of 10g of dried powder was done by using Soxhlet apparatus with the best solvent, optimum particle size, optimum solid-to-solvent ratio and optimum solvent ratio previously determined were employed for 4hours. The samples were filtrated using Wattman paper and evaporated under vacuum. The weight of dry residues was regarded as the content of bioactive components in the roots of the plant.

Maceration extraction (ME)

The best solvent, optimum particle size, optimum solid-to-solvent ratio and optimum solvent ratio were determined then employed in the study of ME. Maceration was performed by continuous constant stirring with cold solvent for 15hours at room temperature. Extracts were concentrated under reduced pressure, dried and stored at 4ºC temperature in an air tight container for further studies. Percentage yield was also calculated.

Global Yield of extraction calculation

The extraction global yield (R) is calculated by the formula below:

$$R\ (\%) = \frac{\text{Mass of the extract obtained}}{\text{Mass of plant material}} \times 100 \quad \text{.....eq.1}$$

Determination of polyphenols and flavonoids content

Total polyphenols content were determined according to the method of Folin-Ciocalteu using Gallic acid standard as described by Kumazawa *et al.*, in 2002 and Singleton *et al.*, in 1999. Absorbance were red at 760nm using a spectrophotometer UV-vis (Shimadzu model). The results are expressed in µg of Gallic acid per mg (GAE) of dry weight plant.

Flavonoids content were evaluated by aluminum chloride colorimetric method (Bahorun *et al*., 1996). Quercetin was used to make the calibration curve. The flavonoids content was expressed in µg per gram of Quercetin equivalent (QE).

DPPH radical scavenging assay

The capacity of methanolic extracts obtained from four extraction techniques to reduce the DPPH (2,2-diphenyl-picrylhydrazyl) radical was assessed using the modified Sánchez-Moreno *et al.* (1998) method. Briefly, the diluted solutions were all prepared in methanol.

Ascorbic acid was used as the standard in solutions ranging from 1 to 100 µg/mL. 0.06 mM DPPH was prepared also in methanol. 1 ml of the solution was mixed with 1 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using spectrophotometer against methanol. The IC_{50} value, which is the concentration at which the test sample reduces 50% of the free radical concentration, was calculated. The blank contained 1 ml of 0.06 mM DPPH solution. The optical density was calculated using the formula:

$$\textit{\% of inhibition of DPPH activity} =: \frac{A-B}{A} X100$$

Where A = optical density of blank and B = optical density of sample.

Statistical analysis

The Statgraphics plus software XVI.II was used for the analysis of variances (Duncan's multiple comparison) of the data obtained at different factor influence.

RESULTS AND DISCUSSION

Effect of Solvent Type on polyphenols and flavonoids Extraction

The correct choice of solvent is fundamental to getting an optimal extraction process. This will depend on the dielectric constant (Ɛ) of the solvent or solvent polarity (Turkmen *et al*., 2006). Three solvents of various polarities i.e. ethyl acetate, ethanol and methanol were used for extracting polyphenols and flavonoids compounds from *X. americana* roots by using MAE. The polarity of the solvents used ranges from low (e.g. ethyl acetate) to high (e.g. methanol) as shown in Table No. 1. The effect of solvent with different types of extraction to

the polyphenols and flavonoids compounds yield from *X. americana* is shown in Table No. 1. The result shows that methanol was the best solvent for polyphenols and flavonoids compounds extraction from the roots of *X. americana*. The total polyphenols and flavonoids amounts recorded in methanol (4992.45 µg GAE /g. DW and 164 µg QE/g.DW) were over threefold higher than that in ethanol (3951 µg GAE/g and 114.32µg QE/g.DW) and ethyl acetate (423.18 µg GAE/g.DW and 10.03 µg QE/g.DW) respectively. The polarity of methanol (0.762) is higher compared to ethanol (0.654) and ethyl acetate (0.228). The total polyphenols and flavonoids extraction from *X. Americana* increasing with increasing polarity, which is in agreement with the findings reported by previous researchers, where methanol was most effective in extracting total polyphenols and flavonoids from grape pomace compared to ethanol (Pinelo *et al.*, 2005). Methanol has a high dielectric constant (Ɛ =32.62) to absorb the microwave energy compare to ethanol and ethyl acetate. The result shows that extraction yield of polyphenols and flavonoids compounds from *X. americana* is greatly affected by the solvent polarity and dielectric constant. Since methanol gave the highest total polyphenols and flavonoids extraction, hence it is used for the remainder of this work.

Table No. 1: Global yield, Polyphenols and Flavonoids compounds in Different Solvent Typed'extraction.

Solvants	dielectric Constant (Ɛ)	Global yield (%)	Total Polyphenols (µg GAE/g.DW)	Total Flavonoids (µg QE /g.DW)
Ethyl acetate	6.50	11.93	423.18 ± 7.05	10.03 ± 1.19
Ethanol	24.70	30.00	3951.33±9.29	114.32±3.22
Methanol	32.62	37.00	4992.45±11.25	164.35 ± 1.18

*Average of three replicated of polyphenols compounds extraction.

[a,b,c,d] Means in the same column followed by different letters are significantly different (P< 0.05) (Newman-Keuls).

Note: µg GAE/g. DW: microgram of gallic acid equivalent per gram of dry weight

µg QE/g. DW: microgram of quercetin equivalent per gram of dry weight

Effect of MAE irradiation time on Polyphenol and flavonoids extraction

Selecting a proper extraction time plays an important factor that not only affects the extraction yield, but also the preservation and stability of polyphenols in plants. In Figure 1, the extraction yield of total polyphenols and total flavonoids increased rapidly with time from 20 to 80 seconds and achieved the optimum yield at 80 seconds with (5046µg GAE/g.DW and 166 µg QE/g.DW). There was a decrease in total polyphenols and flavonoids content at 120 seconds. This can be explained by Fick's second law of diffusion, which predicted that there would be a final equilibrium between the solute in the sample and the extraction solvent after certain of time for a maximal extraction yield of polyphenols (Chew *et al*., 2011). A longer extraction time is not increasing the yield further. In fact, there is a degradation of total polyphenols and flavonoids due to elevated temperature from microwave radiation. The result of this work agrees with Djiobie *et al*., in 2016 who reported that the overexposure in MAE caused degradation of the polyphenols and hence reduction in yield. Therefore, exposure time of 80 seconds was sufficient to obtain the highest total polyphenols and flavonoids from *X. americana* root via MAE.

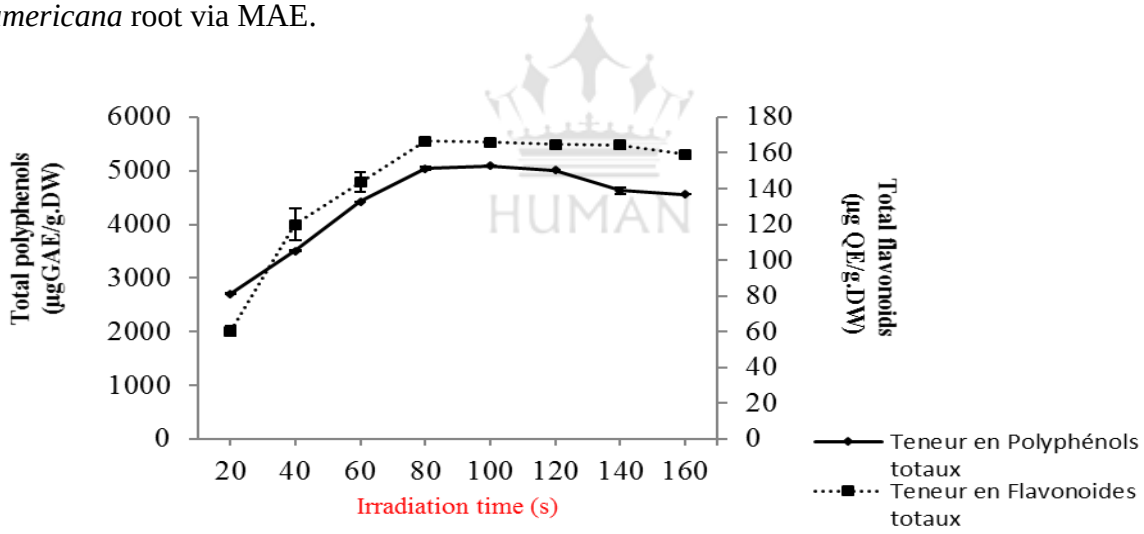

Figure No.1: Total polyphenols and flavonoids in Different Time

Effect of different particle size diameter on Polyphenol and flavonoids extraction in MAE

The total polyphenols and flavonoids extraction from plant material is strongly influenced by the particle size diameter of plant material used (Sparr *et al.*, 2000). For instance, the mass transfer area of smaller particles is greater than that of larger particles, which in turn improves the overall mass transfer process. The dried roots of *X. Americana* was ground,

sieved and classified according to the particle size diameter before the extraction of polyphenols and flavonoids was performed. The roots of *X. Americana* powder consisted of particles with size of 0.2mm, 0.6mm and 1.0 mm. The extraction of total polyphenols and flavonoids showed an increasing trend as the particle size decreases. Total polyphenols and flavonoids obtained using the smallest particle size diameter200µm was the highest which is (5611µg GAE/g.DW and 194µgQE/g.DW) compared to other particle size diameter as shown in Figure fig. 2-a and fig. 2-b. The smaller particles have higher contact surface that enhanced mass transfer, which consequently lead to reduction of extraction time (Silva *et al*., 2007; Cujić *et al*., 2016).

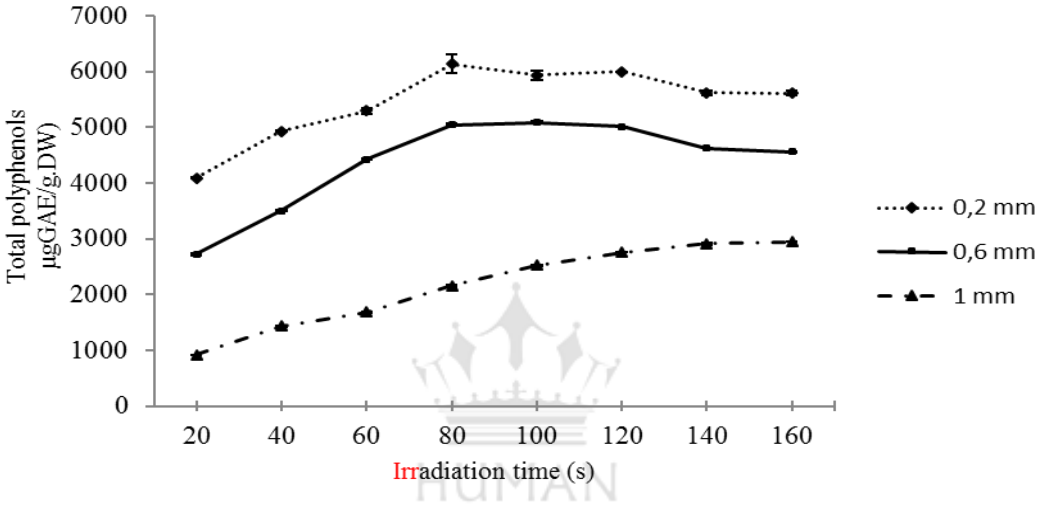

Figure No. 2-a: Total polyphenols in Different Time and in different particle size diameter in MAE

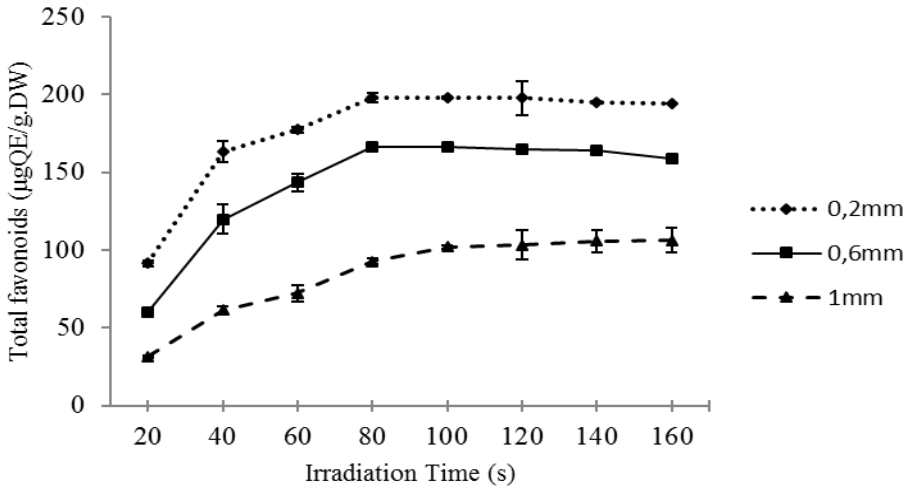

Figure No.2-b: Total flavonoids in different Particle Size Diameter in MAE

Effect of different power on Polyphenol and flavonoids extraction in MAE

Microwave power is critical in softer matrices to avoid degradation of thermal labile compounds (Chin *et al*., 2013). The MAE power affects the irradiation energy supplied to the solvent, which causes rapid direct heating. Figure No. 3 shows that total polyphenols and flavonoids content increases rapidly when power is increased from 200 W to 600 W. However, a marked decrease in total polyphenols and flavonoids content is observed when the power is increased beyond 600 W. Higher power causes a faster molecular movement due to microwave induced dipole rotation and ionic conduction, which in turn cause a rapid increase in temperature. A slight increase in temperature improves the mass transfer and hence facilitates enhancements in extraction yield. However, too much temperature increment at higher MAE power causes a thermal degradation of total polyphenols and flavonoids, hence reducing its content. Li *et al*. (2017) also found that excessive microwave power causes the degradation of some antioxidants, and hence adversely affecting the extraction yield. The highest total polyphenols and flavonoids content (5926 µg GAE/g.DW and 133 µg QE/g.DW) from the roots *X. americana* was achieved at microwave power of 600 W.

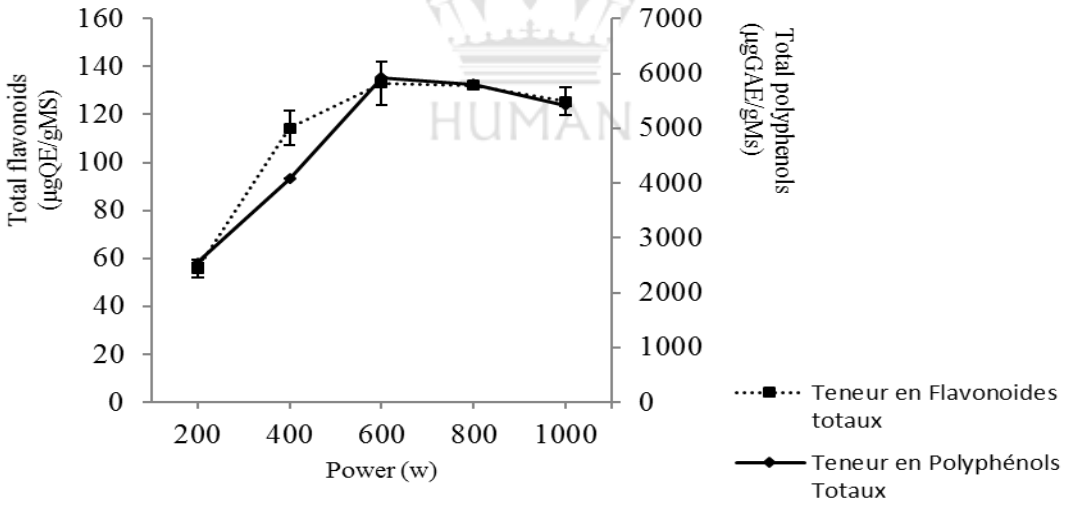

Figure No. 3: Effect of MAE Poweron total polyphenols and flavonoids

Effect of Solvent Ratio on Total Polyphenol and flavonoids Extraction

Aqueous solvent e.g. 70% isopropanol is known to improve polyphenols extraction yield from O. stamineus (Pang *et al*., 2017). Therefore, the extraction yield of methanol at several polarities between (20%-100%) is compared to pure water and pure methanol 100 %. The total polyphenols and flavonoids content increased with increases in methanol content from

20% to 80% as shown in Figure 4. This result agrees with previous work by Bae *et al*.,(2011) who reported a higher total polyphenols and flavonoids obtained using pure methanol compared to aqueous methanol (80%). This is due to the fact that most of the polyphenols presence in polymerized form, which is easily soluble in a moderate polar extraction medium such as methanol. It is known that solvent favour extraction of a similar or closer polyphenols polarity (Yang *et al*., 2008). In this work, polyphenols and flavonoids do not have the same polarity with extraction solvent (pure methanol), and hence easier extracted by binary solvent (80%v/v methanol-water).

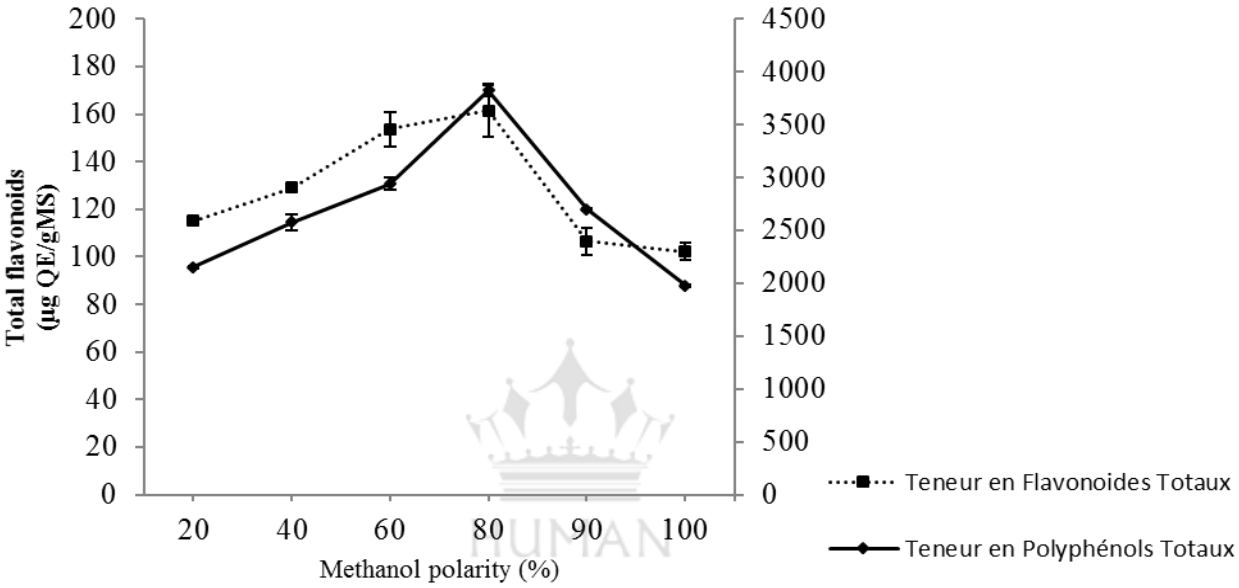

Figure No. 4: Total polyphenols and flavonoids in different methanol ratio in MAE

Total polyphenols and flavonoids in different solvent to solid ratio in MAE

The polyphenols extraction yield is affected by the volume of extraction solvent used (Tan *et al*., 2011). The effect of plant solvent to-solid ratio on the extraction yield of total polyphenols and flavonoids from *X. americana* roots was evaluated by varying the ratio from 5 to 30 mL/g. Figure 6 shows that the yield of total polyphenols and flavonoids increased gradually from solid to solvent ratio of 5 to 20 mL/g which gave the highest yield (3125 µg GAE/g.DW and 138 µg QE/g.DW) but decreased rapidly at 20 mL/g. A higher plant solvent to solid ratio is favourable for polyphenols extraction because it offers a high concentration gradient between the plant material and solute, causing in an increase of diffusion rate of polyphenols into the bulk liquid (Tan *et al*., 2011). However, the amount of total polyphenols and flavonoids from the plant material was limited, and once the concentration of total

polyphenols and flavonoids in the plant material was becoming too low, the extraction yield is no longer increasing with the increase of solvent to plant ratio. The remainder of this work on the comparison of UAE, MAE, SE and ME was performed using a particle diameter of600µm, 80% binary methanol-water solvent and plant solvent to solid ratio of 20 mL/g following the result of the initial screening.

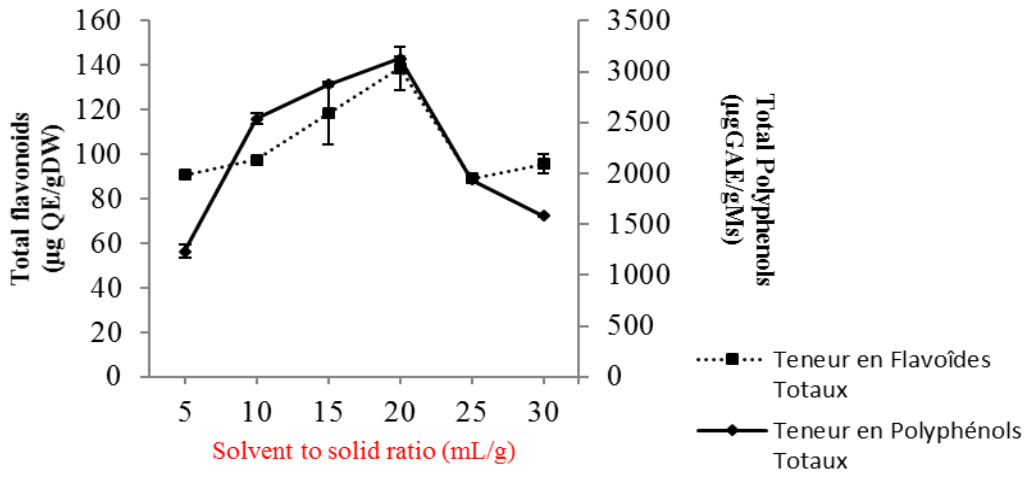

Figure No. 5: Total polyphenols and flavonoids in different solvent to solid ratio

Comparison of UAE, MAE, SE and ME

Four extraction techniques (UAE, MAE, SE and ME) were compared by evaluating the total polyphenols, total flavonoids and antioxidant activity of Ximenia americana root extracts. The experiments were performed on the same sample to solvent ratio (1:20). The result is showed in fig. 6. MAE has the highest extraction yield of total polyphenols and flavonoids (4992.07 µg GAE/g.DW and 164.71 µg QE/g.DW) followed by SE(4839.31µg GAE/g.DW and 141.86 µg QE/g.DW), ME (3136 µg GAE/g.DW and 103.41 µg QE/g.DW) and by UAE (1114.12 µg GAE/g.DW and 15.38 µg QE/g.DW). This shows that conventional method cannot perform an effective extraction of total polyphenols and flavonoids compared to the MAE. Apart from that, ME is time consuming, which required 24 hours compared to 80 seconds for MAE. As mentioned earlier, sonication induces cavitation that causes plant cell wall disruption and allows solvent penetration to the plant matrix resulting in enhancement of polyphenols and flavonoids extraction by the UAE. Meanwhile, the MAE cause a direct rapid heating due to microwave induces molecule dipole rotation and ionic conduction, which cause the cell to break and facilitating the release of total polyphenols and flavonoids to the extracting solvent (Yeong *et al*., 2017). In this work, MAE are the most effective techniques

of extraction of total polyphenols and flavonoids from *X. americana* that gave the highest total polyphenols and flavonoids yield with only 80 seconds compared with ME (15 hours), SE (4 hours) and USE (20 minutes).

A comparison of the antioxidant activity of extracts obtained the four methods MAE, US, So and Ma is also presented in table 2. The IC_{50} is the characteristic value of the antioxidant activity; it gives the concentration corresponding to 50% inhibition. Lower IC_{50} value indicates higher antioxidant activity. It has been noted that the antioxidant activity obtained shows a similar and important antioxidant activity of the extract obtained by ME (IC_{50} = 49.24 ± 3.14 µg/mL) and by MAE (IC_{50} = 55.87 ± 2.21 µg / mL).Compared to the extract obtained by So (IC_{50} = 110.47 ± 2.21 µg / mL), to the extract obtained by the USE (IC_{50} = 204 ± 2.21 µg / mL). The antioxidant properties of aromatic plant extracts are mainly attributed to active compounds present in these plants. This may be due to the high proportion of main constituents, but also to the presence of other constituents in small quantities but with strong activity or synergy between them (Bendaoud *et al*., 2010).

Furthermore, the study of the correlation between this antioxidant activity and the phenolic compounds (TPC and TFC) gave an R^2 of 31.62%, P<0.05 for the Total polyphenols and 79.33%, P<0.05 for the Total flavonoids. This suggested that total polyphenols and total flavonoids are not the only ones responsible for the scavenging activity against DPPH. We can also have tannins, anthocyanins and vitamins. The weak activity in the Soxhlet extract compared to the microwave extract may be due to the destruction or structural change of the flavonoids by prolonged heating of the Soxhlet. These results are in accordance with the correlation coefficient reported by Rolly *et al*., in 2016.

Table No. 2: comparison of different methods on Total polyphenols, total flavonoids and IC_{50} values

Extraction process	Total polyphenols (µg GAE/g.DW)	Total flavonoids (µg QE/g. DW)	IC_{50} (µg/mL)
MAE	4992.07 ± 11.25^{d}	164.71 ± 1.18^{d}	55.87 ± 2.21^{ab}
SE	4839.31 ± 10.35^{c}	141.86 ± 1.02^{c}	110.47 ± 4.08^{c}
USE	1114.12 ± 5.65^{a}	15.38 ± 1.57^{a}	204.00 ± 5.17^{d}
ME	3136.00 ± 9.23^{b}	103.41 ± 1.71^{b}	49.24 ± 3.14^{a}

*Average of three replicated of polyphenols compounds extraction.

[a,b,c,d] Means the column followed by different letters are significantly different (P< 0.05) (Newman-Keuls).

Note: QE: Quercetin equivalent; GAE: Gallic Acid Equivalent; IC_{50}: Concentration of the phenolic compounds that caused 50% Inhibition of DPPH; DPPHsc:2.2-diphenyl-1-picrylhydrazyl; DW: Dry Weight

CONCLUSION

This study showed that particle size of 0.6mm, 80%:v/v binary methanol-water solvent and plant solvent to solid ratio of 20 mL/g gave the highest amount of total polyphenols and flavonoids from *X. americana*. Among the four extraction methods tested, the highest total phenolic and total flavonoid compounds were detected in Microwave extract with maximal values of 4992.07 ± 11.25 µg GAE/g and 161.71±10.18 µgQE/g, followed by Soxhlet extract (4339.31 ± 10.35 µg GAE/gand 141.86±1.02 µgQE/g), maceration extract (3136.00 ± 9.23 µg GAE/gand 103.41±1.71µgQE/g) and finally by ultrasound extract (1114.12 ± 5.65 µg GAE/g and 15.31±1.57µgQE/g). However, the best value of $IC_{50,}$ knowing as the concentration of the phenolic compounds that caused 50% inhibition of DPPHsc was similar and important (IC_{50} = 49.24 ± 3.14 µg/ml) for ME and (IC_{50}= 55.87 ± 2.21 µg / ml) for MAE. A good correlation was observed between this antioxidant activity and the total flavonoids(R^2 =79.33%, P<0.05). This study is the first attempt for recovering antioxidant phenolic compounds by MAE of the roots of *Ximenia americana* and the present findings certainly contribute to ascertain the potential of *Ximenia americana* roots for applications in the food, cosmetic and pharmaceutical industries since antioxidant phenolic compounds could be recovered from *Ximenia americana* and used as a natural and inexpensive alternative to synthetic antioxidants. For completing our work, one perspective it consist in the use of the optimal extraction condition obtained to describe the response surface optimization of solvent extraction of bioactive compounds from the roots of *Ximenia americana* for the enhanced recovery of total phenolic content and flavonoids content with in vitro measuring antioxidant by 2.2-diphenyl-1-picrylhydrazyl free radical-scavenging activity (%DPPHsc).

ACKNOWLEDGEMENT

We acknowledge financial supports from the Ministry of High Education of Cameroon.

REFERENCES

1. Ake AI, Guinko S (1991). In: Plants used in traditional medicine in West Africa. F. Hoffman, La Roche Ltd. Basel Switzerland. p. 100
2. Arbonnier M (2004). Trees, Shrubs and lianas of West African dry zones. Margraf Publishers CIRAD GMBH, MNHN.
3. Bae H, Jayaprakasha GK, Jifon J &Patil BS (2011), Extraction efficiency and validation of an HPLC method for flavonoid analysis in peppers. Food Chemistry 130, 751-758.
4. Bendaoud H., Romdhane M., Souchard J. P., Cazaux S., Bouajila J. (2010). Chemical Composition and Anticancer and Antioxidant Activities of *Schinus Molle L.* and Schinus Terebinthifolius Raddi berries Essential Oils. *Journal of Food Science*;75 (6): 466-472.
5. Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WMW & Ho CW (2011), Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphonstamineous extracts. International food Research Journal 18, 27-35.
6. Chin ChyeTeo • William Pooi Kat Chong • Ying Swan Ho (2013), Development and application of microwave-assisted extraction technique in biological sample preparation for small molecule analysis. Metabolomics (2013) 9:1109-1128 DOI 10.1007/s11306-013-0528-7
7. Cujic N, Savikin K, Jankovic T, Pljevljakusic D, Zdunic G &Ibric S (2016), Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chemistry 194, 135-142.
8. De Sousa, F. A., Guido, C. A. I., Lopes, R. Q. M. E., Teofilo, R. F., Neves, A. A., & de Pinho, G. P. (2012). Evaluation of matrix effect on the GC response of eleven pesticides by PCA. Food Chemistry, 135, 179-185.
9. Djiobie G.T., Tsatsop R .K.T, Kenmogne S. B., Ngassoum M.B., 2016. Optimization of Microwave Assisted Extraction of Bioactive >Compounds from Flavorings Roots of Plant Carissa Edulis. Scholars Journal of Engineering and Technology (SJET). Vol 4(3) PP 127-136.
10. Kenmogne S. B., Ngassoum M, Tchatchueng J. B.nVardamides J.C. and Dongmo A., 2014. Microwave Assisted Extraction of Analgesic Compounds of the Root of *Ximenia americana* (Olacaceae)Research Journal of Chemical Sciences. Vol 4(7), pp 1-6, July.
11. Li Y, Li S, Lin SJ, Zhang JJ, Zhao CN & Li HB (2017), Microwave-assisted extraction of natural antioxidants from the exoticgordoniaaxillaris fruit : optimization and identification of. Phenolics compounds. Molecules 22, 1481.
12. Mapongmetsem P. M., Hamawa Y., Djeumene P., Maissele D., Kossebe C.F., Ndoum J.F., Nduryang J.B., Bebbe F., Bouba D., Wouldata S., Zigro L. & Barbi M. (2008). Valorisation des plantes alimentaires sauvages dans les savanes soudano-guinéennes du Cameroun, In Kapseu, C., Mbofung, C.M., AmvamZollo, P.H. (Eds), Développement de l'agro-industrie et création des richesses (Ngaoundéré, Cameroun, Actes, Conférence Internationale du 9-11juillet 2009, Ngaoundéré, pp. 50-61.
13. Mapongmetsem P. M., Kapchie V. N. &Tefempa B. H. (2012). Diversity of local fruit trees and their contribution in sustaining the rural livelihood in the northern Cameroon. Ethiopian journal of environmental studies and management; 5(1): 32-46.
14. Penchev P.I., Angelov G., Condoret J-S. (2010). "Extraction des agents antioxydants (acide rosmarinique) à partir de la mélisse (*Melissa officinalis L.*)". *Revue de Génie Industriel*; 5: 115-123.
15. Sparr Eskilsson C. &Björklund E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A;* 902(1): 227-250.
16. Silva EM, Rogez H &Larondelle Y (2007), Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. Separation and Purification Technology 55, 381-387.
17. Pang SF, Lau MZ, Yusoff MM &Gimbun J (2017), Microwave irradiation induced fast simultaneous extraction of methoxylated and hydroxylated phenolic compounds from Orthosiphonstamineus leaves. Material Science Forum, 155-158.
18. Yang Y & Zhang F (2008), Ultrasound-assisted extraction of rutin and quercetin from Euonymus alatus (Thunb). Sieb. Ultrasonics Sonochemistry 64, 3-19.
19. Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review.AnalyticalChimicaActa, 703, 8-18.

20. Ogunleye, D.S. and S.F. Ibitoye, 2003. Studies of antimicrobial activity and chemical constituents of Ximenia americana . Trop. J. Pharmacol. Res., 2: 239-241.
21. Onyekwelu NA, Igweh AC, Halid I (2000). Antitrypanosomal and antimicrobial effects of Ximenia americana root extract. Journ. Pharm. Res. Drug Develop. (In press) Abstracts, C6-2.
22. Pinelo M., Rubilar M., Jerez M., Sineiro J., Nunez M.J. (2005). Effect of solvent, Temperature, and solvent to solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace, *Journal of agricultural and food chemistry*; 53(6): 2111-2117.
23. Rolly. K. T. T.; G. T. Djiobie; B. S. Kenmogne; K. R. Regonne; M. B. Ngassoum (2016). Optimization of Microwave-Assisted Extraction of Bioactive Compounds From AnogeissusLeiocarpusGuill. 26 Perr. Stem Bark Using Response Surface Methodology. International Journal of Scientific & Technology Research; (Vol.5, No. 5):103-112.
24. Tadeo, J. L., Sanchez-Brunete, C., Albero, B., & Garcia-Valcarcel, A.I. (2010). Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples. Journal of Chromatography A, 1217, 2415-2440.
25. Tan PW, Tan CP & Ho CW (2011), Antioxidant properties: effect of solid-to-solvent ratio on antioxidant compounds and capacities of pegaga (Centella asiatica). International Food Research Journal 18,553-558.
26. Turkmen N, Sari F &Velioglu YS (2006), Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and FolinCiocalteu methods. Food Chemistry 99, 838-841.
27. Yeong YL, Pang SF, &Gimbun J, (2017), The effect of auxiliary energy on rhein, kaempferol and astragalin extraction from Cassia alata. MATEC Web of Conferences 111, 02009.
28. You, Q., Chen, F., Ni, H., Wang, X., Jiang, Y. M., & McCoy, J. A. H. (2012). HPLC-MS analyses and bioactivities of novel chemicals in devil's club (oplopanaxhorridus (Sm.) Miq.). Food Chemistry, 135, 199-207
29. Zhou, T., Xiao, X., & Li, G. (2012). Microwave accelerated selective soxhlet extraction for the determination of organophosphorus and carbamate pesticides in ginseng with gas chromatography/ mass spectrometry. Analytical Chemistry, 84, 5816-5822.
30. Zhang, L. J., Liu, S. W., Cui, X. Y., Pan, C. P., Zhang, A. L., &Chen, F. (2012). A review of sample preparation methods for the pesticide residue analysis in foods. Central European Journal of Chemistry, 10, 900-925.