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Study of the Effect of Different Operating Parameters on the Microwave – Assisted Extraction of Phenolic Compound from the Roots of *Sarcocephalus latifolius (sm.) Bruce*

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ABSTRACT

The plant species used in this work is *Sarcocephalus latifolius (sm.) bruce*. It is a medicinal plant widely used in traditional herbal medicine to treat several diseases such as fever, pains, dental caries and malaria. The influence of various operating parameters (time and microwave power, solvent polarity, solid to liquid ratio) on the extraction of phenolic compounds from the roots of *Sarcocephalus latifolius* (sm.) bruce has been studied. This allowed us to choose the most appropriate operating conditions for this system for realistic applications. Three techniques were evaluated: microwave-assisted, soxhlet, and maceration extraction. The results show that the optimal extraction conditions are: time 80 sec (833.9 µg GAE/g DM and 625.15 µg QE/g DM), power 70 w (702.48 µg GAE/g DM and 612.64 µg QE /g DM), solvent volume 80 mL (788,64 µg GAE /g DM and 372 µg QE /g DM) and a 60 % v/ v water ratio (751.83 µg QE /g DM and 372.01µg GAE/g DM) for polyphenols (PT) and total flavonoids (FT). The comparative study carried out on three extraction methods reveals that the microwave extract has the highest value of PT and FT (4852.14 µg GAE /g MS, 598.05 µg QE /g DM), followed by the Soxhlet extract (3652.10 µg GAE /g DM, 556.35 µg QE /g MS) and finally the maceration extract (2950.33 µg GAE/g DM, 382 µg QE/g DM). It has been noted also that the antioxidant activity obtained by microwave (IC_{50} = 24.97 ± 0.86 µg/mL) was more important than that of maceration (IC_{50} = 32.62 ± 0.34 µg/mL) and soxhlet methods (IC_{50} = 61.15 ± 1.88 µg / mL). The results obtained in this work highlight the influence of microwave operating parameters and the extraction method used on the phenolic compounds content of the Roots of *Sarcocephalus Latifolius (Sm.) Bruce*.

INTRODUCTION

Sarcocephalus latifolius (Sm.) Bruce, commonly known as Africa peach, is a plant belonging to the genus Sarcocephalus in the Rubiaceae family [1]. It's a wild plant found in the northern parts of Cameroon and all of sub-Saharan Africa [2]. In folk medicine, it has been used for the treatment of fever, pains, dental caries, malaria, convulsions, and epilepsy, just to name a few [3]. Different studies carried out on this plant have demonstrated that it has proven antibacterial, antiradial, antisalmonella, antimicrobial, antimalarial, anti-inflammatory, antioxidant, and antidiabetic activities[3-6]. The presence of various phénolic compounds in *Sarcocephalus latifolius (Sm.) Bruce*, such as polyphénols and flavonoids, which comprise gallic acid, ferulic acid, keamferol, catechin, quercetin, and rutin is responsible for those different biological activities [7]. Phenolic compound are typically obtained by extraction from the plant. Due to the polar structure of the phenolic compounds, they are highly soluble in water and alcohol [8]. Therefore, various solvents, including methanol and ethanol with different proportions of water, have been used for the extraction of phenolic compound from different plants. The conventional or traditional extraction techniques are less efficient and time consuming [9]. The novel extraction techniques, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE), can significantly improve the efficiency of phenolic compound extraction [10]. Kenmogne et *al*., 2014[11] have reported that the MAE reduces extraction time and solvent consumption and improves extraction yield. However, studies involving the MAE of phenolic compounds from the roots of *Sarcocephalus latifolius (Sm.) bruce* can still be hardly found, especially the effects of operating extraction parameters such as microwave irradiation time and power, solid-to-liquid ratio, and polarity of solvent.

I. MATERIALS AND METHODS

II.1. Plant material

The roots of *Sarcocephalus latifolius (Sm.) bruce* were collected in Cameroon more precisely in Ngaoundédé on April 05, 2021. The taxonomic identification was made by the botanist Nana Victor or a specimen was deposited in the national herbarium under the reference No. 5285/ SRF/Cam.

II.2.Study of the influence of the extraction parameters on the TPT and the TFT of the extracts obtained by microwave of *Sarcocephalus latifolius (Sm.) bruce.*

This step is to determine the best extraction conditions of the total polyphenolic and total flavonoid contained in the roots of *Sarcocephus latifolius (SM) Bruce* by using the microwave-assisted extraction method. The experimental model is divided into two parts. A preliminary test is performed to determine the types of factors that affect the response of interest, as well as their ups and upstream levels, depending on the classic extraction method by varies a parameter and setting the others [12].

II.2.1. Influence of time on microwave-assisted extraction

In a 250 mL flask containing 50 mL of methanol, introduce 5 g of the root powder of *Sarcocephalus latifolius (Sm.) bruce.* After 10 sec of extraction at 30 w in the microwave oven model WBFY-201, the solution was filtered using Whatman paper then the filtrate obtained was concentrated using a rotary evaporator. The extract was then recovered in a labelled bottle and stored. This experiment is repeated at different times such as: 20, 40, 60, 80, 100, 120 sec with a power fixed at 30 w, the ratio of 1/10 w/v.

II.2.2. Influence of power on microwave-assisted extraction

Into a 250 mL flask containing 5 g of powder, introduce 50 mL methanol. After 60 sec of extraction at a power of 10 Watt in the microwave oven model WBFY-201, the solution was filtered using Whatman paper then the filtrate obtained was concentrated using an evaporator rotary. The extract was then recovered in a labelled bottle and stored. This experiment is repeated at different powers such as: 10, 30, 50, 70, 90 with the time fixed at 60 sec, the ratio at 1/10.

II.2.3. Influence of solid/liquid ratio on microwave-assisted extraction

In a 250_mL flask containing 5 g of root powder, introduce 50 mL of methanol. After 60 sec of extraction at 30 W in the microwave oven model WBFY-201, the solution was filtered using Whatman paper then the filtrate obtained was concentrated using a rotary evaporator. The extract was then recovered in a labelled bottle and stored. This experiment is repeated at different

volumes such as: 20, 40, 60, 80, 100, 120 mL with a power set at 30W, time at 60 sec and mass at 5g.

II.2.4. Influence of solvent concentration on microwave-assisted extraction

In a 250 mL flask containing 5 g of powder, introduce 50 mL of methanol. After 60 sec of extraction at 30 W in a model WBFY-202 microwave oven, the solution was filtered using Whatman paper and then the filtrate obtained was concentrated using a rotary evaporator. The extract was then recovered in a labelled bottle and stored. The extraction was carried out using a hydro alcoholic solvent (Methanol / Water) at different concentrations such as: 20 %, 40 %, 60 %, 80 %, 100 % (v / v) with the time set at 60 sec, the power at 30 w, the mass at 5 g and the volume of the solvent at 50 mL.

III.3. Comparative study of extraction methods

III. 3.1. Obtaining extracts

III. 3.1.1. Microwave assisted extraction

In a 200 mL beaker, 10 g of powder were introduced into 100 mL of solvent. After 2 minutes of extraction in the LG domestic microwave oven, the solution was filtered using Wattman paper and then the filtrate obtained was concentrated using a rotary evaporator.

III. 3.1.2. Extraction by maceration

5 g of the powder was introduced into 50 mL of methanol and left to stand for 24 hours at room temperature. Then a filtration was done. The filtrate obtained is concentrated using a JANKE & JUNNEL (IKA-WERK) rotary evaporator and we will obtain our crude extract. A weighing at this stage allows a calculation of the extract yield.

III.3.1.3. Soxhlet extraction

Introduce 25 g of *Sarcocephalus latifolius (Sm.) bruce* root powder. Into the cellulose cartridge, then into the soxhlet reservoir. Introduce 250 mL of solvent into the flask (take into account the quantity that will be trapped in the tank during manipulation) and overcome the condenser

extractor. Using a heating flask, bring the solvent to a boil. At the end of the extraction, the contents of the flask are filtered and the filtrate is concentrated using a rotary evaporator.

III.3.1.4. Assays of total polyphenols content

The determination of total polyphenols was done according to the method described by (Soto and *al*., 2014) [13]. A volume of 0.5 ml of extract was added to 1 ml of Folin Ciocalteu's reagent diluted to (1/10 v/v). After 4 minutes of incubation in the dark, 0.8 mL of sodium carbonate (75 %) is added. The absorbance was measured with a spectrophotometer at 700 nm after 30 minutes of incubation. The concentration of total polyphenols was calculated from the regression equation of the calibration curve, established with the standard gallic acid standard and expressed in microgram equivalents of gallic acid per gram of dry matter (µg EGA/ g DM).

III.3.1.5. Assays of total flavonoids content

The dosage of flavonoids is based on the formation of a very stable complex between aluminium chloride ($AlCl_3$) and the hydroxide groups (OH) of phenols, which has a yellow color whose intensity is proportional to the amount of flavonoids present in the extract. The quantification of flavonoids is carried out by the aluminium trichloride method adapted by TliLi et *al*., 2013[14] with some modifications. It consists of mixing 750 µL of extract and 750µL of 2 % aluminium chloride. The mixture is left to react for 15 min in the dark then the absorbance is read at 420 nm. The concentration of flavonoids in the extracts is calculated from the calibration curve established with quercetin.

IV. RESULTS AND DISCUSSION

IV.1. Study of the influence of the extraction parameters on the TPC and the TFC of the extracts obtained by microwave of *Sarcocephalus latifolius (Sm.) bruce*.

The interest of this study is to determine the influence of the parameters on the extraction of polyphenols and total flavonoids from the roots *of Sarcocephalus latifolius (Sm.) bruce.*

V.1.1. Study of the influence of time on the content of total polyphenols and flavonoids

The extraction time is a main parameter in the extraction procedure of phenolic compounds, it can vary from seconds to hours [15]. Figures 1 above give the values of the evolution of the TPC and the TFC as a function of time.

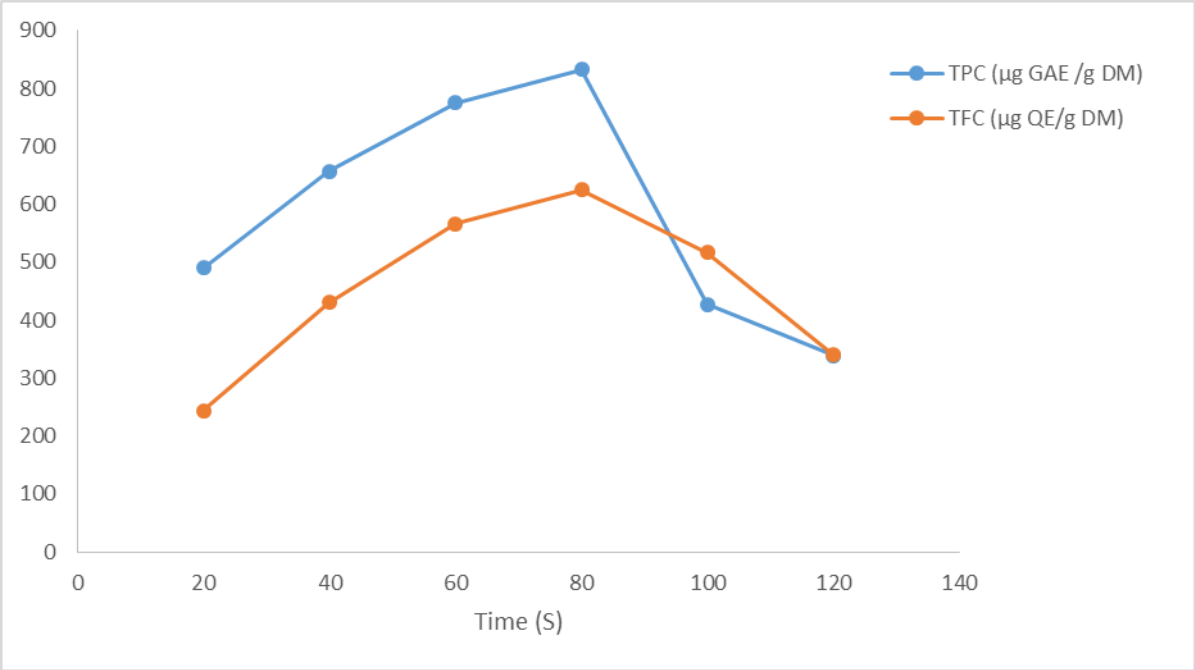

Figure 1: Total polyphenols and flavonoids content as a function of time (Power: 30W, Ratio: 1/10 w/v, Volume: 50 mL, mass: 5 g)

In view of this table, it appears that the TPC and the TFC increase with a time varying from 20 to 80 sec and beyond 80 sec, the TPC decreases. These obtained results confirm the principle of Fick's second diffusion law which states that: "After a certain time, the solute concentration in the plant matrix decreases so that a final equilibrium is reached". Thus, a long enough extraction time does not always increase the extraction yield. In addition, the transfer speed is thus decreasing until it reaches a zero minimum corresponding to the exhaustion of the bark of the trunk. This decrease would be caused by the degradation and oxidation of polyphenol compounds following microwave irradiation at prolonged extraction times [16]. The result of this work agrees with Djiobie et *al.*, in 2016 [16] who reported that the over exposure in microwave assisted extraction 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 root of *Sarcocephalus latifolius (Sm.) bruce* via microwave assisted extraction.

V. 1.2. Study of the influence of power on the content of total polyphenols and flavonoids

The selection of extraction power is one of the important steps in optimization. The increase in power improves the extraction of polyphenols, by making the cell membranes more permeable,

and increases the solubility and the diffusion coefficient of the compounds to be extracted and it decreases the viscosity of the solvent which facilitates its passage into the substrates solid. Strong sturdy sound robust firm secure durable substantial tough hard rugged reliable steady stout tight safe fast stiff stern massy forte serviceable able-bodied well-built cast iron. [17]

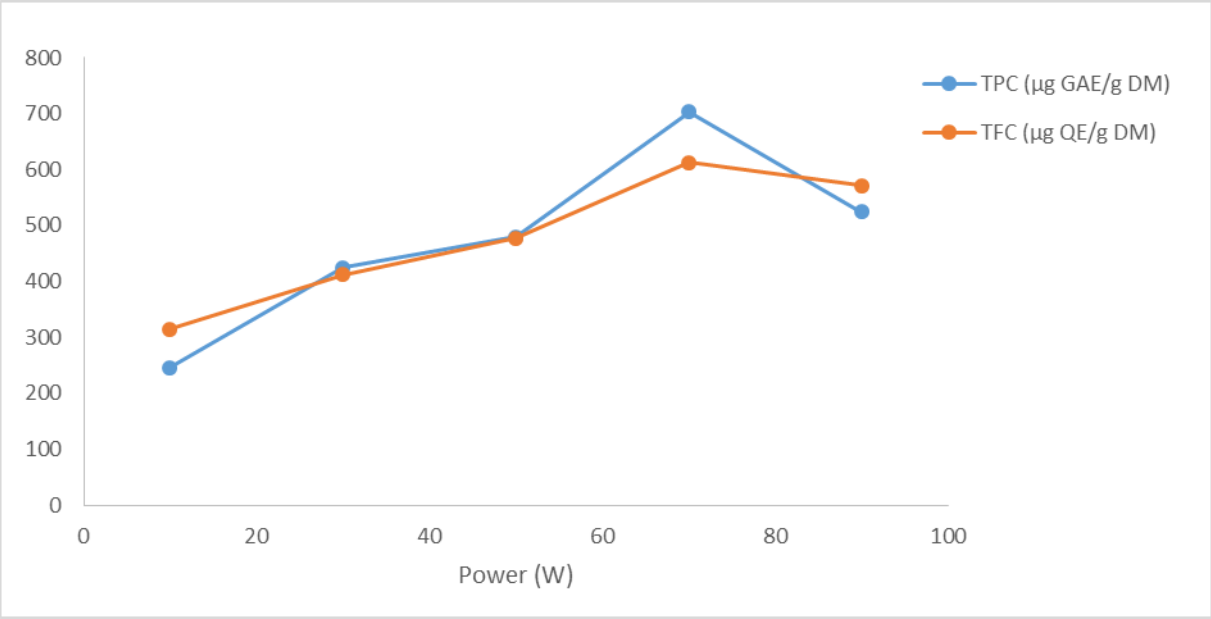

Figure 2: Total polyphenol and flavonoids content as a function of power (Time: 60 sec, Ratio: 1/10 w/v, Volume: 50ml, mass: 5 g)

Figure 2 above give the values of the evolution of the TPC and the TFC as a function of the power. With regard to this table, it appears that the TPC and the TFC increase with a power varying from 10 to 70 w and beyond 70 w, the content decreases considerably. We obtain a maximum value at 70 w for polyphenols and flavonoids. This is related to the effect of microwave power on the bark matrix. Indeed, the power of the microwaves improves the extraction by improving the transfer of matter through the following actions: the damage of the membranes and cell walls of the solid and the improvement of the penetration of the solvent and the solubility of the compounds present in the solid thanks to the high temperatures and pressures in the cavitation zone, enhancing the diffusion process while allowing fresh or less charged solvent to enter the layer and the extracted compounds [18]. However, excessive potencies can

cause the degradation of phenolic compounds, or it can cause a decrease in the selectivity of the extraction, which limits the extraction potencies to a certain the hold [19].

The highest total polyphenols and flavonoids content from the roots of *Sarcocephalus latifolius (Sm.) Bruce* was achieved at microwave power of 70 w.

V. 1.3. Study of the influence of the ratio on the content of total polyphenols and flavonoids.

The results of the effect of the ratio on the extraction of phenolic compounds are represented by the figure below. Figure 3 show the evolution of the content of polyphenols and total flavonoids according to different volumes.

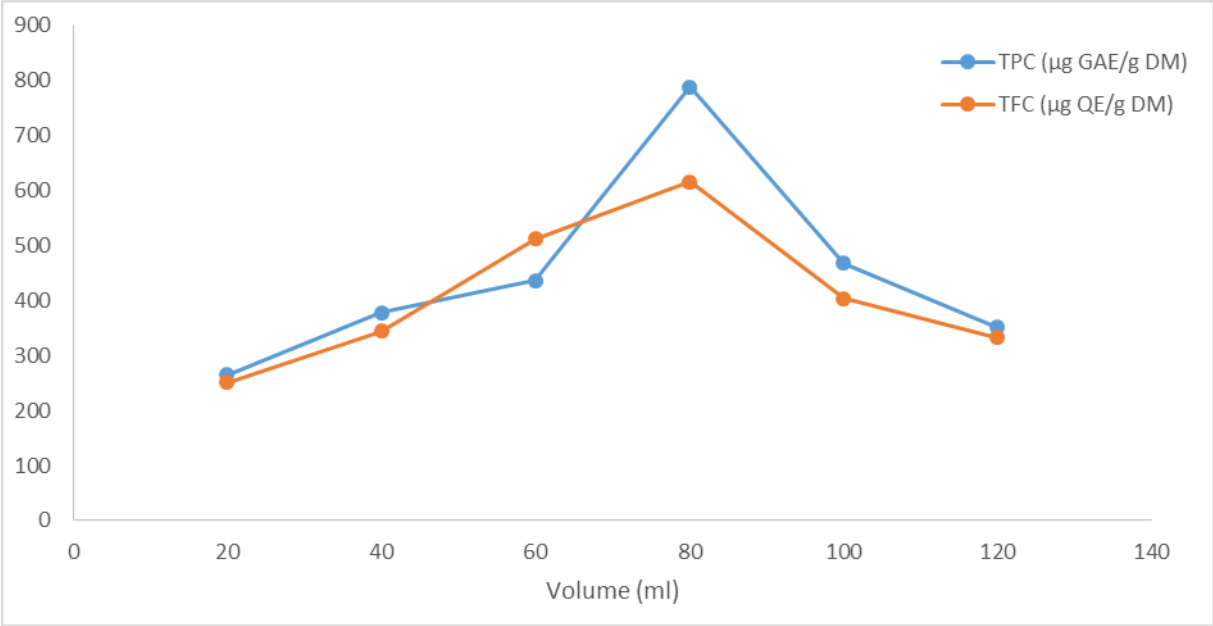

Figure 3: Total polyphenols and flavonoids content as a function of solvent volume (Power: 30 w, Time: 60 sec, mass: 5 g)

We observe that the higher the solvent volume, the better the extraction of polyphenols and flavonoids. The maximum content that can be obtained in the extract is 788.64 µg GAE/g DM for polyphenols and 616.3 µg QE/g DM for flavonoids, it is obtained with a volume of 80 mL. Beyond 80 mL, the content of polyphenols and flavonoids drops considerably. According to the principles of mass transfer, the driving force during the transfer is the concentration gradient between the solid and the liquid medium. This concentration gradient is large when the liquid-solid ratio is high. On the other hand, increasing the amount of solvent beyond a certain value, no

longer has any effect on the driving force because the limitation of mass transfer is due to the limitation of intra-particle mass transfer, which leads to a decrease in the content of phenolic compounds. (Agbangnan et *al*., 2013)[20].

V. 1.4. Study of the influence of solvent concentration on the content of total polyphenols and flavonoids

Regarding the influence of water concentration, the selection of extraction solvent is critical as it will determine the amount and type of phenolic compounds extracted [21]. The solvent-distilled water mixture seems very effective for the extraction of polyphenols, since the water in combination with the methanol contributes to an increase in polarity which ensures both the extraction of the phenolic compounds and the preservation of their antioxidant activity.

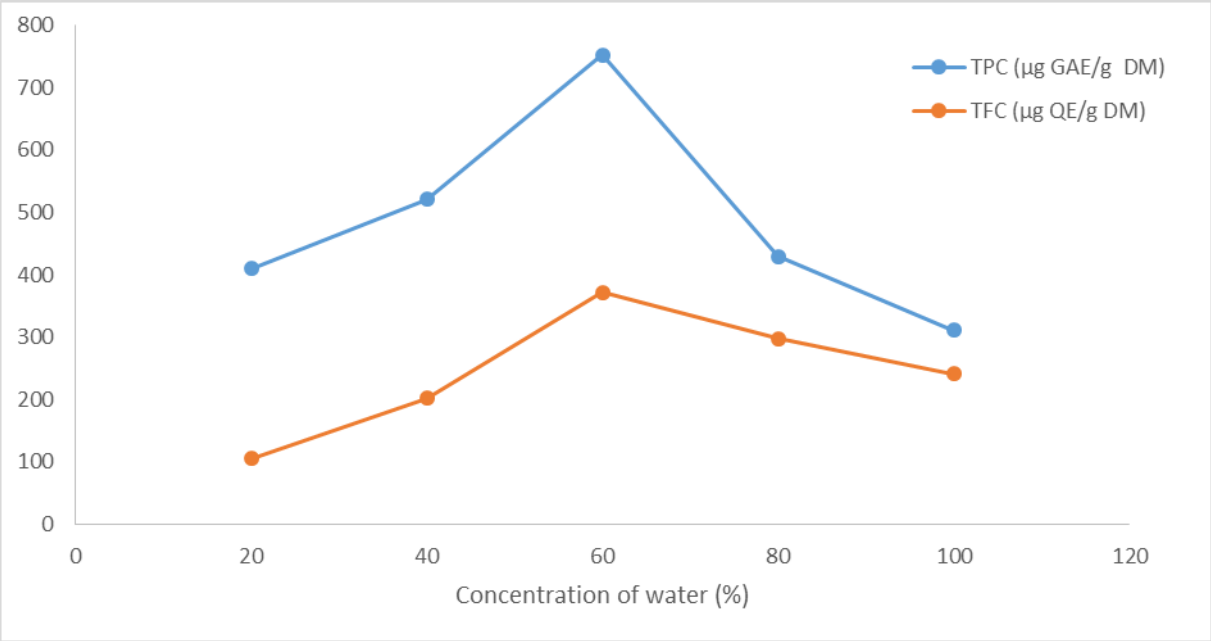

Figure 4: Total polyphenol content as a function of water concentration (Power: 30 w, Ratio: 1/10 w/v, Volume: 50 mL, mass: 5 g)

As shown in Figures 4, the TPC and TFC in methanol (20% **v/v**) is lower than in water (90% **v/v**). This result may be due to the higher solubility of PT and FT in a solvent with a high amount of water, this is particularly evident in the field of microwave-assisted extraction. TPC and TFC increase with water concentration and then decrease after reaching a maximum of 60% with increasing concentration. Therefore, the capacity of methanol extraction is variable in a

microwave-assisted extraction system. The constant and the dielectric loss factor are higher for water than for methanol. Under the same microwave irradiation power intensity, water absorbs more energy than methanol and thus promotes the extraction of polyphenols and total flavonoids from the core of the matrix from the inside out. However, the driving force decreases with increasing methanol polarity. The total polyphenols extracted from the roots of *Sarcocephalus latifolius (Sm.) bruce* have a large number of polar hydroxyl groups. The increase in the polarity of methanol results in the addition of polar molecules close to those of polyphenols (increase in the polarity of the medium). Thus, the solubility of total flavonoids can also be improved with the polarity of methanol based on the principle that similar substances are more likely to be dissolved. This is because methanol plays an important role in breaking the hydrogen and hydrophobic bonds that exist between phenolic compounds and the plant matrix cell. (Xu et *al*., 2007)[22]. For a methanol polarity varying from 60 – 90% **v/v**, a reduction of the TPC and the TFC is observed due to the increase in the polarity of the methanol. The diffusion coefficient decreases with the low solubility of polyphenols and total flavonoids in alcohol than in water.

VI.1. Comparative study of methods for extracting compounds with antioxidant.
In this study, we used three extraction methods: maceration, microwave-assisted extraction and soxhlet extraction. In total, we obtained three dry extracts with which the different tests were carried out. The comparative study of these three extraction methods focused on: The extraction yield, the quantitative dosage of polyphenols and total flavonoids.

VI.1.1. Determination of phenolic compounds in *Sarcocephalus latifolius (Sm.) bruce* root extracts.

Phenolic compounds are used to prevent several diseases which are mainly associated with free radicals. More generally, phenolic compounds have been recognized as antioxidant agents, which slow down degradation due to the effects of oxidation ensuring better aging, and therefore exhibiting medicinal activity and physiological functions (Rawat et al., 2011)[23]. The content of phenolic compounds in extracts of *Sarcocephalus latifolius (Sm.) Bruce* was determined and the results are summarized in Table 1. The concentration of total polyphenols is expressed in microgram of gallic acid equivalent per gram of plant dry matter (µg GAE/g DM) and that of total flavonoids is expressed in microgram of quercetin equivalent per gram of dry matter (µg QE/g DM), it is a function the extraction method used.

Table 1: Polyphenol and total flavonoid content of *Sarcocephalus latifolius (Sm.) Bruce* root extracts.

Extraction methods	Total polyphenol content (µg GAE/g DM)	Total flavonoid content (µg QE/ g DM)
Microwave extraction	852.14 ± 5.89	598.05 ± 3.45
Soxhlet extraction	652.10 ± 10.09	556.35 ± 7.09
Macération extraction	250.33 ± 7.17	382.67 ± 3.11

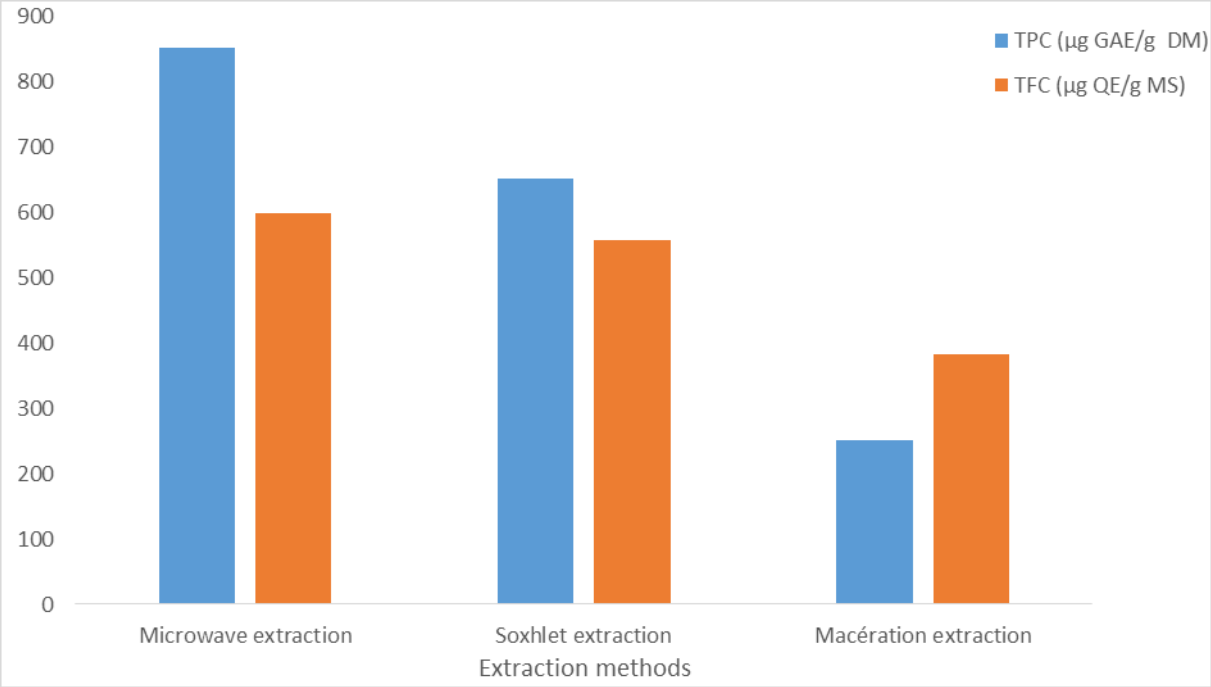

Figure 5: Total polyphenol and flavonoids content as a function of extraction methods (Power: 30 w, Ratio: 1/10, Volume: 50 mL, mass: 5 g)

The results which are represented in Table 5 indicate that the Total polyphenol content obtained by the various techniques varies from 250.33 to 852.14 µg GAE/g DM and 382.67 to 598.05 µg QE/ g DM) from total polyphenols and flavonoids content respectively. The highest value (852.14 µg GAE /g DM and 598.05 µg QE / g DM) results from the extraction obtained by microwave, while the lowest content (250.33 µg EAG/g DM and 382.67 µg QE / g DM) is obtained with the maceration extract. Mustapa et *al*., (2015)[24] observed the same result when extracting phenolic compounds from *Clinacanthus nut ans* with this method, this is due to the

diffusion of secondary metabolites which results from the increase in permeability. During extraction, the transferred mass increases due to the high penetrating capacity following cell destruction. And that the absorption energy of the polyphenols by the solvent with the microwave-assisted extraction is much higher compared to the extraction by soxhlet and maceration due to the long extraction time that these methods take to reach the balance and so that the polyphenols are completely extracted [24]. The values obtained by microwave-assisted extraction (852.14 µg GAE/g DM and 598.05 µg QE/ g DM) in 80 seconds are higher than those of the extract obtained by Soxhlet (652.10 µg GAE /g DM and 556.35 µg QE / g DM) in 6 hours and by maceration (250.33µg GAE/g DM and 250.33 µg QE/ g DM) in 24h. These results confirm the effectiveness of the new extraction technique and its many advantages over traditional techniques. The result obtained by the microwave-assisted extraction was confirmed by the studies carried out by Rolly et *al.*, 2016 [10] which show that the extraction contents of the phenolic compounds of black chokeberry are obtained after 15 min of extraction compared to the other extractions due to strong diffusion of the compounds in the solvent and the power of the microwave waves to demolish the structure of the vegetable matter, which subsequently increases the penetration of solvent which favours the progression of the extraction rates, unlike maceration and soxhlet (methods conventional).

VI.1.2. Evaluation of antioxidant activity

A comparison of the antioxidant activity of extracts obtained the three extraction methods such as microwave, soxhlet and maceration is also presented in table 2.

Table 2: antioxidant activity of *Sarcocephalus latifolius* (Sm.) Bruce root extracts of the different extraction methods

Extraction methods	**Microwave**	**Soxhlet**	**Maceration**	**Vitamin C (IC_{50} (µg/mL))**
DPPH (IC_{50} (µg/mL)	24.97 ± 0.86	61.15 ± 1.88	32.62 ± 0.34	7.80 ± 1.24

*The results obtained by the different extraction methods are an average of 3 repetitions ± standard deviation

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 by microwave (IC_{50} = 24.97 ± 0.86 µg/mL) is more important than maceration (IC_{50} = 32.62 ± 0.34 µg/mL) and soxhlet methods (IC_{50} = 61.15 ± 1.88 µg / mL). The antioxidant properties of Sarcocephalus *latifolius (Sm.) bruce* extracts are mainly attributed to active aromatic 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) [25].

* TPC: total polyphenol content, TFC: Total flavonoids content, QE: Quercetin equivalent; GAE: Gallic Acid Equivalent; IC_{50}: Concentration of the phenolic compounds that caused 50% Inhibition of DPPH; DPPH: 2.2-diphenyl-1picrylhydrazyl; DM: Dry Matter

CONCLUSION

This study showed that 80%:v/v binary methanol-water solvent, solid to liquid ratio 5g / 80ml, time 80 sec and the power 70 w of gave the highest amount of total polyphenols and flavonoids from *Sarcocephalus latifolius (Sm.) bruce*. Among the three extraction methods tested, the highest total phenolic and total flavonoid compounds were detected in Microwave extract with maximal values (852.14 µg GAE /g DM and 250.33 µg GAE/g DM) followed by Soxhlet extract (652.10 µg GAE/g DM and 556,35 µg QE/ g DM), maceration extract (250.33µg GAE/g DM and 250.33 µg QE/ g DM). 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}) for microwave assisted extraction. This study is the first attempt for recovering antioxidant phenolic compounds by microwave assisted extraction of the roots of *Sarcocephalus latifolius (Sm.) bruce* 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 ***S****arcocephalus latifolius (Sm.) bruce* and used as a natural and inexpensive alternative to synthetic antioxidants. For completing our work, one perspective it consists 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 *Sarcocephalus latifolius (Sm.) bruce* 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 (%DPPH).

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