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Microwave-Assisted Extraction of Bioactive Compounds from *Combretum micranthum* Bark: Process Optimization

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ABSTRACT

This study compared three extraction methods (microwave, Soxhlet, and maceration) for phytochemical compounds present in the trunk bark of *Combretum micranthum*. Following extraction, a phytochemical study was conducted to determine the different families of compounds present in the plant. The results revealed that the plant contains polyphenols and alkaloids. Furthermore, a comparative study of the yields obtained from the three extraction methods showed that microwave-assisted extraction with methanol gave the best yield, at 7.34%, unlike conventional methods, which gave lower yields, at 3.15% for maceration and 3.80% for Soxhlet. Regarding the content of phenolic compounds, we obtained values of 7.465 mg GAE/g extract for microwave, 2.980 mg GAE/g extract for Soxhlet, and 2.460 mg GAE/g extract for maceration. Additionally, antibacterial tests conducted on nine strains using the microdilution method showed that some strains are sensitive while others are resistant, depending on the extraction method used. The extracts obtained by Soxhlet showed the highest antibacterial activities, suggesting that the extraction method influences the results of antibacterial tests. Furthermore, antioxidant tests were conducted using three methods: DPPH, ABTS, and FRAP. The results showed that the maceration extract gave the best antioxidant activity, with the lowest RSA50 values, notably 20.83 ± 3.11c µg/mL for DPPH, 23.11 ± 1.20b µg/mL for ABTS, and 21.56 ± 0.53b for FRAP. Moreover, regarding modeling, the determination coefficient gave a value of 99.17%, indicating that the model explains the variance well and can be used to predict the content of phenolic compounds. Optimal extraction conditions were obtained when the time was at a high level (60 s), the power was at a high level (50 W), and the ratio was at a low level (0.025 g/mL).

Keywords: extraction methods, bioactive compounds, bark, *Combretum micranthum*, microwave, optimization.

INTRODUCTION

Photosynthesis is a set of reactions that enable plants (algae, plants) to convert light energy into chemical energy usable for the synthesis of organic matter (Houcine et al., 2013). Generally, the extraction of bioactive substances from a plant, such as *Combretum micranthum*, prior to chemical analysis, consists of two stages: extraction and analysis. While the analytical stage typically requires a few minutes, the extraction stage can take several hours, as is the case with the Soxhlet method, which is the reference extraction procedure. This technique is a classical method for solid-liquid extraction and allows for continuous extraction of a chemical species contained in a powder using a solvent (AOAC, 2019). Although highly effective and widely accepted by the scientific community, Soxhlet extraction has several significant drawbacks, including long extraction procedures and large solvent consumption (Penchev et al., 2010). To address this limitation, American engineer Percy Spencer, inspired by a chocolate bar melting in his pocket while working on an active radar, developed a modern solid-liquid extraction technique: microwave-assisted extraction. This method appears to be an interesting alternative, as it allows for reduced solvent use, shorter processing times, higher yields, and better selectivity (Kenmogne et al., 2014). However, the challenge lies in finding the extraction conditions for bioactive molecules that meet these requirements, enabling the formulation of a medication. Therefore, the theme of this research work is titled "Microwave-Assisted Extraction of Bioactive Compounds from *Combretum micranthum* Bark: Process Optimization." Specifically, this study aims to compare conventional and modern methods and find the optimal extraction conditions for bioactive compounds from *Combretum micranthum* bark.

Material

The plant material that has permitted to extract the secondary metabolites is the bark of the trunk of the Combretum Micranthum plant recess in Cameroon in the locality of Bandja on July 10, 2023 and dried away from the sun for 14 days Then reduced powder

for the help of an electrical device. son botanical identification has been confirmed by m. Nana Roger Taxonomist at the Plant Biology Laboratory of the University of Yaoundé 2.

Method

1.Determination of the moisture content (MC)

The test of the water content is carried out to determine the amount of water existing in the fresh plant. To determine the water content of the plant, a precise amount of the bark of Combretum micranthum is weighed immediately after the harvest and is set to the oven at room temperature.This operation is carried out until the mass becomes constant then according to (AOAC, 2000) the following formula can be applied:

$$MC(\%) = \frac{(Mwb - Mdb) * 100}{Mwb}$$

MC: moisture content ; Mwb : mass of wet bark ; Mdb : mass of dry bark.

2. Extraction

The extracts obtained from the powder have been prepared according to the conventional (Soxhlet and maceration) and modern (microwave) processes this has allowed the different extracts to know the extract to the maceration, to the Soxhlet and in the microwave.

2-1-maceration

The maceration consists in immersing 50 g of powder from the bark of the *Combretum Micranthum* in 500 ml of solvent for 8 hours. Then, the filtration is performed on filter paper and the solvent was recovered from the filtrate by evaporation in a steam rota. The resulting solution is dried in the ventilated oven (45 ° C) for 24 hours to obtain a powder or paw that is stored at 4 °C until use (Khosravi et al., 2013).

2-2-Soxhlet

introduce 15g of powder from the bark *Combretum Micranthum* The cellulose cartridge, then in the Soxhlet tank. Insert in the 150 ml of solvent (take into account the quantity that will be trapped in the tank during handling) and overcome the refrigerant extractor. With the help of a balloon heater, the solvent is carried on to a boil. At the end of the extraction, the contents of the balloon and the filtrate are filtered up with a rotary evaporator. (AOAC 2019)

2-3-microwave

in a 500 ml flask, 5 g of powder were introduced into 50 ml of solvent. After 60 seconds of extraction in the LG brand domestic microwave oven, the solution was filtered using the Wattman paper and the filtrate obtained was concentrated using a rotary evaporator. The extract was then retrieved in a labeled bottle and then preserved. (Kenmogne and coworkers., 2014)

3-Determination of the extraction yield

The weight of the dry extract is determined by the difference between the weight of the full balloon (after evaporation) and the weight of the empty balloon (before the transfer of the filtrate to be evaporated (Mohammedi, 2005).

yield (%) = (Dry weight of extract * 100 / (weight of plant material)

4-Detection of secondary metabolites

The purpose of these tests is the highlighting of the secondary metabolites present in the different extracts.

4-1-highlighting polyphenols (reaction FeCl3)

2 ml of each solution in a test tube and add a few drops of FeCl3 to 10% .The presence of polyphenols in the extracts will be indicated by the appearance of the blackish green color.

4-2 -Missed flavonoids

The highlighting of flavonoids was carried out according to the protocol described by Bekro and coworkers.2007) by making some modifications. 2 ml of each aqueous phase obtained after each extraction method, have been placed in a test tube containing hydrochloric alcohol (4 ml ethanol + 1ml concentrated HCl). After adding 2 or 3 magnesium chips and the addition of 3 drops of isoamyl alcohol, the intensification of a pink-orange or purplish coloring indicated the presence of flavonoids.

5-Spectrophometric Dosage Total Polyphenols

The polyphenol assay is carried out according to the method described by Soto and *al* 2014 using the Folin-Ciocalteu reagent. This reagent consists of a mixture of phosphotungic acid and phosphomolybdic acid. It is reduced during the oxidation of phenols in a mixture of blue tungsten oxide and molybdenum.

5-1-operating mode

A volume of 0.1 ml of each extract is mixed with1 ml of the folin-ciocalteu reagent; After 4 min, add 0.8 ml of the 2% sodium carbonate solution; The whole thing is stirred by a vortex The resulting mixture is incubated at room temperature and sheltered from light for 30 minutes the playback is performed against a white using a 699nm spectrophotometer. A standard curve is made in parallel under the same operating conditions using gallic acid as standard at different concentrations.

6-Assessment of biological activities

6-1-Evaluation of antibacterial

activities to assess the antimicrobial activity of the *Combretum Micranthum* extract. We used the microdilution method in the agar medium. This method has exactly the same principle as that of antibiogram tests, that is to say the application of disks impregnated with active ingredients on crop media seeded with microorganisms. The antimicrobial activity, when it is present, is then manifested by zones of inhibition around the discs.

6-1-1-Midfielder Culture

The culture media used for the realization of antibacterial tests is the Mueller Hinton agar for the study of the sensitivity of bacteria at the extract of the plant (CLSI, 2012).

6-1-2-Bacterial strains

The bacterial strains chosen for this study are pathogenic bacteria frequently involved in contamination and alteration of food.

6-1-3-Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) is defined as the smallest dilution in which no macroscopic growth is observed (Kuete and coworkers., 2018).

6-1-4-ICM Determination Protocol

The muller's moodilution control method has been used for bacterial species sensitivity tests in 96-well microtiter sterile plates as described by Newton and coworkers.,(2002) .For the raw extracts were dissolved in a 5% DMSO solution and then dill with mueller broth to obtain a mother concentration of (2000 µg / ml). Serial dilution of twice samples were made with Mueller Hinton broth to give a volume of 100 µl / well this gave a concentration range of 1000 to 0.96 µg / ml.cent microliters of inoculum bacterial (containing about 1.5 x 106 UFC / mL) were added to the respective wells containing samples and meagerly melted to give final concentrations ranging from 500 to 0.48 µg / ml .The plates of MicroDilution were incubated for 24 hours the minimum inhibitory concentrations were detected after addition of 50 µl of 0.2 mg / ml of p-iodonitrotetrazolium chloride (INT). The lowest concentration to which no color change has been observed has been considered Comm Minimal Bactericidal Concentration (CMB) -the minimum bactericidal (CMB) concentration corresponds to the lowest essential oil concentration or gross extract capable of killing more than 99.9% of the initial bacterial inoculum (less than 0.01% survivors).

6-1-5-MBC determination protocol

The bactericid concentrations have been determined by adding aliquots of 50 µl of the preparations (without int), which showed no visible color changes after transplanting from MIC tests, in 150 µl broth Mueller Hinton without extract. These preparations were further incubated at 37 ° C for 48 hours and bacterial growth was revealed by the addition of int as above. The smallest concentration to which no color change has been observed has been considered the MBC. The tests were made in duplicate. The MBC / MIC ratio was calculated to determine the bactericidal effects (MBC / MIC ≤ 4) and bacteriostatic (MBC / MIC> 4.

6-2-Evaluation of antifungal activities

6-2-1-preparation of inoculums of Levures

The levure inoculums have been prepared from 48h elderly crops by taking many colonies and suspending them in a sterile saline (NaCl) solution at 0.9%. The absorbance was read at 530 nm and adjusted with the saline solution to correspond to that of a standard 0.5 mcfarland solution, corresponding to about 106 louval cells / ml.

6-2-2- DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM FUNGICIDAL CONCENTRATION (MFC)

6-2-2-1-Determination of the minimum inhibitory concentration

The minimum inhibitory concentration of each extract has been determined using broth microdilution techniques in accordance with the guidelines of "Clinical and Laboratory Standards Institute" (CLSI, TrainLy National Committee for Clinical and Laboratory Standards, NCCLS) for louvures (M27-A2), (CLSI, 2008). Mother solutions test extracts were prepared in a 5% and diluted DMSO solution with dextrose tall broth (BSD) to give a concentration of 1 mg / ml. This has been diluted in series twice to obtain a concentration page of 500 to 0.24 µg / ml for extracts and 125 to 0.24 µg / ml for isolated compounds. The final concentration of DMSO in the well was less than 0.5% (Loba Chemie, India). The negative control well consisted of 150 µL of BSD and 50 µl (containing about 106 cells / ml for louvure) of the inoculum. The plates were covered with a sterile and incubated lid on the agitator at 37 ° C for 48 hours (for louvures). The CMIs were visually evaluated after the corresponding incubation period and were considered the lowest to which there was no growth or virtually no crossance.

6-2-2-2-Minimum fungicide concentration (CMF).

The test was repeated three times. For the determination of the CMPs, the 50 µl aliquots of each well that showed no microorganism growth were replicated in 150 µL of BSD and incubated at 37 ° C for 48 hours (louvures). The lowest concentration that gave no growth after subculture has been taken as NYStatin's CMF (for louvures) used as positive controls.

6-3-Evaluation of antioxidant

activity Many tests are used to evaluate the antioxidant activity of extracts. Most of these tests are based on the coloring or discoloration of a reagent in the reaction medium. Our study is based on three tests: The DPPH, Framp, Edits test.

6-3-1-Test of trapping of radicals DPPH

6-3-1-1-Principle

The principle is based on the capacity of the compounds contained in the extracts to provide protons with free radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH radical is unstable and when it reacts with an antioxidant compound, which can give hydrogen ions, it is reduced and becomes stable. This reducer power of the extract is revealed by a color change of the purple to yellow. A decrease in the absorbance at 517 nm is proportional to the antioxidant potential of extracts. Trapping free radicals of CM extracts were evaluated using the DPPH analysis as described by (Koleva et *al.*2002) the trapping activities of the raw extract radicals were evaluated by Spectrophotometer using the 1,1diphenyl-2-picrylhydrayl free radical (DPPH). When the DPPH reacts with an antioxidant compound, which can give hydrogen, it is reduced. Color changes have been measured at a wavelength of 517 nm under UV Spectrophotometer / Visible Light (Infinite M200, Tecan, Swiss). The extract (1000 ug / ml) was diluted twice in series with methanol. Fifty microliters of the diluted extract (1000 µg / ml) in methanol were mixed with 150 µL of 0.02% methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), giving a final concentration range from from 250 to 1.9531 µg / ml (250, 125, 62.5, 31.25, 15,625, 7,8125, 3,9062 and 1,9531 µg / ml). After 30 minutes of incubation in the dark at room temperature, the optical density was measured. Ascorbic acid (vitamin C) has been used as a positive control. Each dosage was made in triplicate and the results, saved as an average ± standard deviation (SD). The trapping activity of the radicals (RSA,%) was calculated as follows: the percentages

of trapping of the radicals were plotted according to the logarithmic values of the concentration of the test samples and a linear regression curve has been established to calculate the RSA50 or IC50, which is the concentration of the sample required to reduce the total Radical Total DPPH.

6-3-2- ABTS trapping test

This test was made with slight modifications of the method described by (Arnao, 2000).

6-3-2-1-Principle

The radical abts + is formed by a loss of an election of the nitrogen atom at the presence of potassium permanganate (KMNO4) or potassium persulfate (K2S2O8). The principle is based on the capacity of the compounds contained in the extracts to reduce the blue-green 2, 2'-azino bis (3-ethylbenzothiazoline-6-sulfonic) (abts +) with colorless abts by donating his h +. This assay makes it possible to measure the antioxidant capacity of the lipophilic and hydrophilic compounds in the sample (Iqbal and *al*., 2007). The discoloration of the radical measured by 734 Nm spectrophotometry is proportional to the concentration of antioxidant of extracts.

6-3-2-2-Procedure

The AbTS method is known to be a quick method for determining the antioxidant activity and could be a useful tool for screening samples to achieve high natural antioxidant content (Silva et al., 2006). The trapping activities of the radicals of the crude extract were evaluated by spectrophotometry using the free radical of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (abts) When Disputes reacts with an antioxidant compound, which can give hydrogen, it is reduced. Color changes were measured at 734 nm under UV / Visible Light Spectrophotometer (Infinite M200 (Tecan, Swiss)). Pure methanol has been used to calibrate the meter. The extract (1000 ug / ml) was diluted twice in series with methanol. Twenty-five microliters of the diluted extract were mixed with sixty-shaped µl of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (3-ethylbenzothiazoline-6-sulfonic acid solution (), to give a final concentration range of Extract from 250 - 1,9531 µg / ml (250, 125, 62.5, 31,25, 15,625, 7,8125, 3,9062 and 1,9531 µg / ml). After 30 minutes of incubation in the dark at room temperature, the optical densities were measured at 734 nm. Ascorbic acid (vitamin C) has been used as a control. Each test was made in triple and the results, recorded as an average ± standard deviation (SD) of the three results, were presented as a table. The radical trapping activity (RSA, in%) was calculated as follows: the percentages of trapping of the radicals were plotted according to the logarithmic values of concentration of the test samples and a linear regression curve A Established to calculate the RSA50 or IC50, which is the concentration of the sample required to lower the total abts radical.

6-3-3-Iron Reduction Test: FERRIC Reducing Power Antioxidant (FRAP).

The assay was carried out according to the method described by Bassène, 2012 with slight modifications.

6-3-3-1-Principle

The principle is based on the reduction of Fe3 + in Fe2 + by components of the extract, which in the presence of ortho-Phenanthroline forms a brown or orange red color complex. The complex absorbs 505 nm and the intensity of the coloring is proportional to the amount of FE3 + converted by the extract.

6-3-3-2-Procedure

The ferric reduction potential (Fe3 + conversion potential in Fe2 +) of Kinkeliba extracts was determined according to the method described by. (Padmaja and al., 2001). Briefly, the extracts were first dissolved as for the DPPH dosage. 25 µl of each dilution were introduced into a new microplate and 25 µL of 1.2 mg / ml of FE3 + solution were added. The plates were pre-incubated for 15 minutes at room temperature. After this time, 50 µL of 0.2% orthenanthroline were added to obtain final concentrations of Extract of 250, 125, 62.5, 31,25, 15,625, 7,8125, 3,90625 and 1,95325 µg / ml. The reaction mixtures were further incubated for 15 min at room temperature, after which the absorbance was measured at 505 nm under UV / visible light spectrophotometer (Infinite M200 Tecan, Switzerland) against the white (consisting of 25 µl of methanol + 25 µl Fe3 + + 50 µL ortho-phenanthroline). Ascorbic acid (vitamin C) has been used as a positive control. The dosage was made in triplicate. From the OB (optical density), reduction percentages were calculated for each concentration © and used to determine the RC50 from the dose-response curves.

7- Optimization of Extraction Conditions

7-1 Preliminary Studies

Preliminary studies were conducted to determine the best conditions for extracting phenolic compounds using microwave-assisted extraction by varying parameters such as extraction time, extraction power, and solid-liquid ratio (H. Taleb Née Bouderaa, 2015).

7-1-1 Type of Extraction Solvent

Microwave-assisted extraction was performed using methanol and ethyl acetate, both in their pure state. The time was set to 1 minute, and the power was set to 50 W. The best solvent was chosen based on the highest total polyphenol content (expressed in mg GAE/g of powder).

7-1-2 Extraction Time

Extraction was performed using the optimized solvent and subjecting the extracts to the defined power, with varying time (10, 20, 30, 40, 50, 60 seconds). The best extraction period was chosen based on the highest total phenolic compound content.

7-1-3 Microwave Power

A determined weight of Combretum micranthum powder was mixed with 50 mL of the optimized solvent and exposed to different microwave powers (10 W, 20 W, 30 W, 40 W, 50 W, 60 W). The optimal power was defined based on the highest extracted phenolic compound content.

7-1-4 Solid-Liquid Ratio

The determination of the solid-liquid ratio was performed by fixing the mass of the solid material (3 g of powder) and varying the liquid phase (20, 40, 60, 80, 100, 120 mL). Other parameters were fixed at previously optimized values.

7-1-5 Application to Experimental Design

Optimization of the extraction method was performed using a full factorial design at two levels to evaluate the combined effect of three independent variables: extraction time, extraction power, and solid-liquid ratio, designated as T, P, and R, respectively. Preliminary studies allowed for the determination of low and high levels for the parameters influencing experimentation. These parameters were studied to optimize the response in total phenolic compounds. According to the formula N = 2^K, 8 trials were conducted to estimate the mathematical model of the investigated response, where N is the number of trials, K is the number of factors, and 2 is the number of levels per factor.

The response surface methodology allows modeling the studied response in the form of a first-degree polynomial equation presented below:

$$Y = a0 + a_T T + a_P P + a_R R + a_{TP} TP + a_{TR} TR + a_{PR} PR + a_{TPR} TPR + E \text{ (Montgomery, 2012)}$$

Where a0 is the response value at the center of the study domain, noted 0; ai is the effect of factor i; aij is the effect of the interaction between factors i and j; T, P, R are the studied factors; Y is the measured response (yield of total phenolic compounds); and E is the error.

Results table

Table 1 : Results of secondary metabolite yields analysis

Extraction techniques	Ratio (g/ml)	Solvents used	Extraction time	total extract yield
Microwave	5/50	Methanol	1 minute	7,340%
		Ethyl Acetate		1,600%
Soxhlet	15/150	Methanol	3 hours	3,800%
		Ethyl Acetate		0,700%
Maceration	50/500	Mtheanol	8 hours	3,152%
		Ethyl Acetate		0,592%

Table 2 : phytochemical screening results

Metabolites	Maceration	Soxhlet		Microwave
Polyphenols	++	++		+++
Flavonoids	–		–	–
Quinones	–		–	–
Alkaloids	++	++		+++

+++ Abundant ; ++ moderate ; + trace amounts ; – Absent

Table 3 : Results of total polyphenol Assays

Extraction techniques	Solvents used	Total polyphenol content (mgGAE/g E)
Microwave	Methanol	7,465
	Ethyl Acetate	1,660
Soxhlet	Methanol	2,980
	Ethyl Acetate	0,290
Maceration	Methanol	2,460
	Ethyl Acetate	0, 145

Table 4: Antibacterial test results

Species	préparation method	Bacterial strains	MBC (µg/ml)	MIC (µg/ml)	MBC/MIC	Antibacterial activity
COMBRETUM MICRANTHUM	MACERATION	*Pseudomonas aeruginosa (PA)*	>500	500	ND	
		Staphylococcus aureus (SA)	500	250	2	BACTERICIDAL
		Escherishia coli (EC)	>500	500	ND	
		Klessiella pneumonae(KP)	>500	500	ND	
		Shigella flexineri (SF)	>500	500	ND	
		Shigella dysenteria (SD)	500	250	2	BACTERICIDAL
		Salmonella typhimurium(Stm)	>500	500	ND	
		Salmonella typhi (ST)	>500	500	ND	
		Salmonella enteritidis (SE)	500	250	2	BACTERICIDAL
	SOXHLET	*Pseudomonas aeruginosa (PA)*	500	250	2	BACTERICIDAL
		Staphylococcus aureus (SA)	>500	500	ND	
		Escherishia coli (EC)	250	125	2	BACTERICIDAL
		Klessiella pneumonae(KP)	>500	500	ND	
		Shigella flexineri (SF)	500	250	2	BACTERICIDAL
		Shigella dysenteria (SD)	>500	500	ND	
		Salmonella typhimurium(Stm)	>500	500	ND	

		Salmonella typhi (ST)	500	250	2	BACTERICIDAL
		Salmonella enteritidis (SE	>500	500	ND	
	MICROWAVE					
		Pseudomonas aeruginosa (PA)	500	250	2	BACTERICIDAL
		Staphylococcus aureus (SA)	>500	500	ND	
		Escherishia coli (EC)	>500	500	ND	
		Klessiella pneumonae(KP)	>500	500	ND	
		Shigella flexineri (SF)	>500	500	ND	
		Shigella dysenteria (SD)	500	250	2	BACTERICIDAL
		Salmonella typhimurium(Stm)	>500	500	ND	
		Salmonella typhi (ST)	>500	500	ND	
		Salmonella enteritidis (SE	>500	500	ND	

MBC : minimum bactericidal concentration ; MIC : minimum inhibitory concentration ND : no detected

Table 5 :Antifungal test results

Species	préparation method	Bacterial strains		MFC (µg/ml)	MIC (µg/ml)	MFC/MIC	Antifungal activity
	MACERATION	*Candida albicans (CA)*	Yeasts	500	250	2	FUNGICIDE
		Candida krusei (CK)		ND	>500	ND	
		Candida parasilosis (CP)		250	125	2	FUNGICIDE
		Cryptococcus néoformans (CN)		>500	500	ND	
		Trichophyton mentagrophytes(TM)	Dermatophytes	>500	500	ND	
		Microsporium audouinii (MA)		>500	500	ND	
		Epidermophyton flocosum (EF)		>500	500	ND	
	SOXHLET	*Candida albicans (CA)*	Yeasts	>500	500	ND	
		Candida krusei (CK)		250	125	2	FUNGICIDE
		Candida parasilosis (CP)		>500	500	ND	
		Cryptococcus néoformans (CN)		>500	500	ND	
COMBRETUM MICRANTHUM		Trichophyton mentagrophytes(TM)	Dermatophytes	500	250	2	FUNGICIDE
		Microsporium audouinii (MA)		>500	500	ND	

		Epidermophyton flocosum (EF)		500	250	2	FUNGICIDE
	MICROWAVE						
		Candida albicans (CA)	Yeasts	500	250	2	FUNGICIDE
		Candida krusei (CK)		>500	500	ND	
		Candida parasilosis (CP)		>500	500	ND	
		Cryptococcus néoformans (CN)		>500	500	ND	
		Trichophyton mentagrophytes(TM)	Dermatophytes	>500	500	ND	
		Microsporium audouinii (MA)		>500	500	ND	
		Epidermophyton flocosum (EF)		>500	500	ND	

MIC :Minimum inhibitory concentration ; MFC : Minimum fungicidal concentration ND : No detected

Table 6 : Antioxydant activity results

Tested sample	DPPH (**RSa_{50} (µg/mL)**)	ABTS (**RSa_{50} (µg/mL)**)	FRAP (**RC_{50} (µg/mL)**)
CM maceration	20.83 ± 3.11^{c}	23.11 ± 1.20^{b}	21.56 ± 0.53^{b}
CM microwave	35.24 ± 1.73^{d}	25.87 ± 0.74^{c}	21.66 ± 0.71^{b}
CM soxhlet	33.64 ± 0.21^{c}	73.49 ± 3.48^{d}	41.25 ± 6.05^{d}

C M : *combretum micranthum*

Table 7 : Independent variables and levels of factors influencing microwave-assisted extraction

Factors	**Parameters**	**Units**	**Low level -1**	**High level+1**
T	Extraction time	Second (s)	40	60
P	Extraction power	Watt (W)	30	50
R	Solid-liquid ratio	g /ml	3/120	3/80

Table 8 : Results of full factorial design

Test number	t(s)	P (W)	R(g/ml)	Y_{exp}(mgEAG/g E)
01	−1	−1	−1	0,7530
02	−1	−1	+1	0,8740
03	−1	+1	−1	2,8620
04	−1	+1	+1	2,8180
05	+1	−1	−1	3,6690
06	+1	−1	+1	3,8280
07	+1	+1	−1	4,3330
08	+1	+1	+1	3,4160

Table 9 : Coefficients and second-order interactions of the model

Coefficient	a_0	aT	aP	aR	aTP	aTR	aPR
Values	-19,08000	0,34150	0,36890	169,00000	-0,00475	-1,67000	-2,48000

Table 10 : Analysis of variance(Anova) of the statistical model

SOURCE	DL	Somcar	CM ajust	F-Value	P-Value
Linear	3	10,2531	3,41769	32,94	0,073
T	1	7,8785	7,87847	75,94	0,003
P	1	2,3166	2,31663	22,33	0,013
R	1	0,0580	0,05797	0,56	0,591
Two-factor interaction	3	2,0856	0,69520	6,70	0,075
T*P	1	1,8060	1,80595	17,41	0,150
T*R	1	0,0872	0,08715	0,84	0,008
P*R	1	0,1925	0,19251	1,86	0,403
Erreur	1	0,1035	0,10374		
Total	7	12,4424			
R^2 =99,17%					
R2ajut=94,16%					

Table 11: Optimal extraction conditions for polyphenols

Optimal condition	Estimation
Extraction time (t)	60 (s)
Power (P)	50 (w)
Solid-liquid ratio (R)	0,025 (g/mL)

Figure 1 : Leaves of *combretum micranthum*

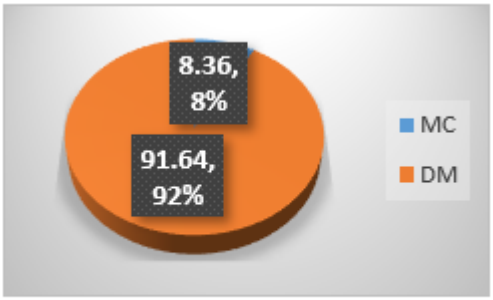

Figure 2 : Moisture content and dry matter

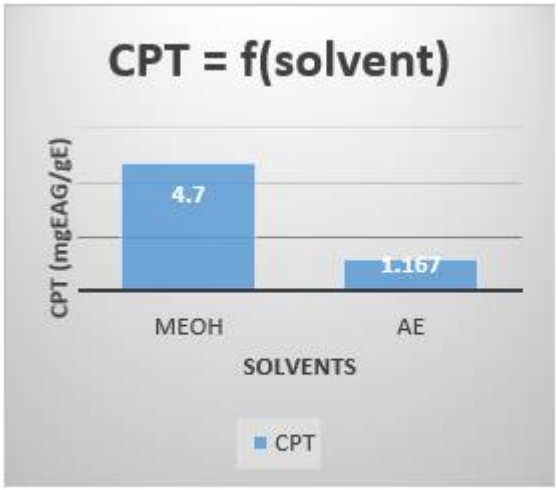

Figure 3 :The effect of solvent on the yield of total phenols

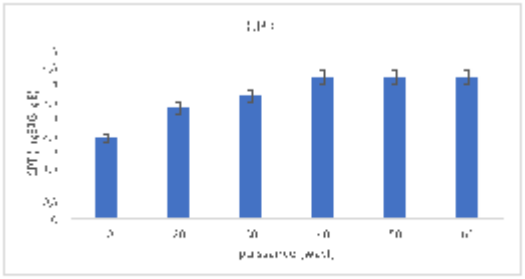

Figure 4 : Effect of microwave power on polyphenol extraction

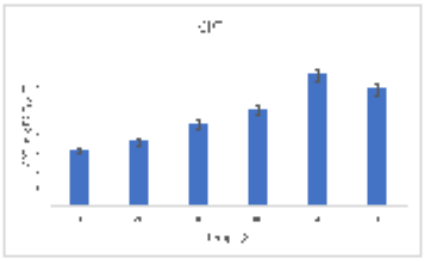

Figure 5: Effect of time on polyphenol extraction

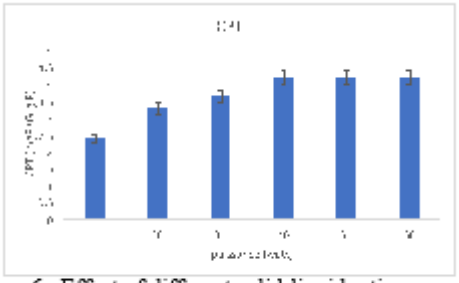

Figure 6 : Effect of different solid-liquid ratios on polyphenol extraction

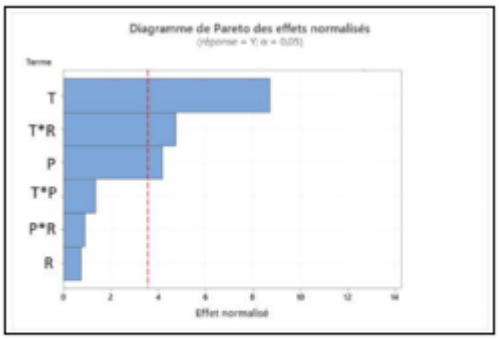

Figure 7 : Pareto diagram showing the standardized influence of Each effect

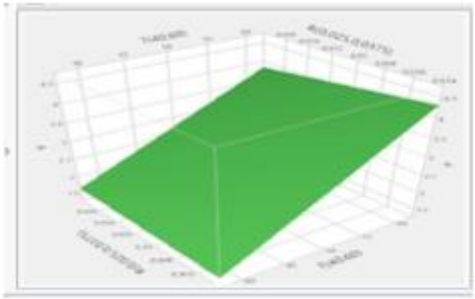

Figure 8 : Influence of time-ratio interaction on phenolic compound response

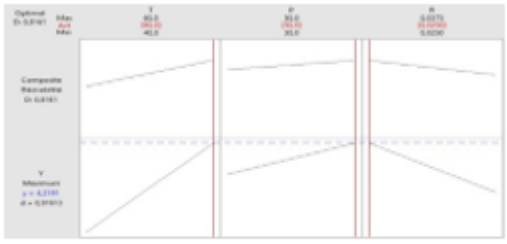

Figure 9 : Optimization curve for phenolic compound

Figure 10 : combretum micranthum Bark

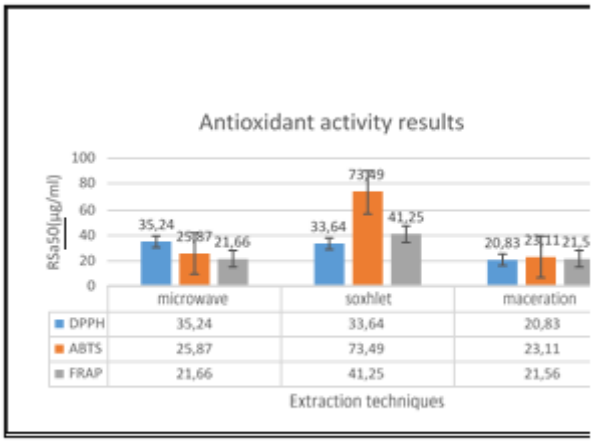

Figure 1 : Antioxidant activity results

Discussion

1-Moisture content

The water content of the studied plant gave us a value less than 10% or 8.36% which is favorable to a good preservation of the interest molecules. A high water content (> 10%) promotes oxidation, fermentation and mold development reactions that are prejudicial to the quality of the active ingredient. (Camara., 2017).

2-Global extraction

yield Table 1 shows that the microwave extract released in a minute 7.340% of polar molecules and 1,600% medium polar compounds. For what is from the soxhlet extract, we note a Release of 3,800% polar compounds and 0.700% molecular medium polar in three hours of time. In addition to the maceration extract showed a release of 3.152% of polar molecules and 0.592% molecules moderately polar for 8 hours of time. It is apparent from these results that microwave extraction has been made in such a short time and gave the best efficiency whatever the solvent used more, the *Combretum Micranthum* plant is richer in strong molecules In moderately polar molecule this result concords with the studies of Kenmogne and coworkers 2014 which demonstrate that the biggest advantage of microwave assisted extraction of the phenolic compounds of the *Combretum Micranthum* bark compared to Soxhlet is the drastic reduction of the volume of solvent and extraction time.

3-Phytochemical tests

The preliminary evaluation of the phytochemical composition of _*Combretum micranthum*_ bark revealed the presence of several chemical families, as presented in Table 2. The results of the phytochemical screening conducted on the bark of _*Combretum micranthum*_, as shown in Table 2, indicate the absence of quinones and flavonoids in all three extraction methods. The comprehensive study of the phytochemical screening highlights the presence of other chemical compounds with interesting biological activities, notably polyphenolic substances (tannins and saponins). Alkaloids were found to be abundant in the microwave extraction method, while moderate presence of alkaloids and polyphenols was observed in the maceration and Soxhlet methods.The nature of the chemical constituents revealed by the phytochemical screening suggests potential pharmacological activities of the plant bark. Our results partially corroborate with those of Etame-Loe and coworkers. (2018), who observed the absence of alkaloids and the presence of some phenolic compounds in trace amounts, as well as the absence of flavonoids, which is consistent with our findings. The differences in results may be attributed to environmental, geographical, and ecological differences in the harvesting locations .Indeed, according to the World Health Organization's guidelines on good agricultural and collection practices for medicinal plants, the same plant harvested from different sites can exhibit differences in composition due to varying climatic, geographical, and ecological conditions, as well as differences in soil composition and environment (WHO, 2012).

4- polyphenol essay

Table 3 presents the results of the total phenolic compound assay of *Combretum micranthum* bark. It is observed that three extraction methods were experimented using ethyl acetate and methanol. The results showed that microwave-assisted extraction yielded the highest concentration of polyphenols regardless of the solvent used. Furthermore, the concentration was significantly higher with methanol, which demonstrates that *Combretum micranthum* bark is rich in polar bioactive compounds. These results corroborate with those of Kenmogne and coworkers. (2014).

5-Interpretation of antibacterial tests

Antibacterial tests were conducted on three preparation methods, namely Soxhlet, maceration, and microwave. For the Soxhlet extract, a weak activity was observed for the strains *_Pseudomonas aeruginosa_ (PA), _Staphylococcus aureus_ (SA), _Klebsiella pneumoniae_ (KP), _Shigella flexneri_ (SF), _Shigella dysenteriae_ (SD), _Salmonella typhi_* (St), *_Salmonella typhimurium_* (Stm), and *_Salmonella enteritidis_ (Se).* The most significant activities were observed on the strains *_Pseudomonas aeruginosa_ (PA), _Shigella flexneri_* (SF), and *_Salmonella typhimurium_* (St), with a MIC of 250 µg/ml. Additionally, a moderate activity was observed on the strain *_Escherichia coli_* (EC), with a MIC of 125 µg/ml. Therefore, the extract is bactericidal against the strains *PA, EC, SF*, and *St*, with a MBC/MIC ratio of 4.The microwave extract showed a weak activity against all strains, with the most significant activities observed on the strains *PA* and *SD*, with a MBC/MIC ratio of 4. The maceration extract showed a weak activity against the 9 tested strains, with the most significant activities observed on the strains *SA, SD*, and *Se,* with a MBC of 250 µg/ml. The extracts from the different extraction methods were tolerant against the strains *_Klebsiella pneumoniae_* and *_Salmonella typhimurium_*.The results show that the Soxhlet extraction method was bactericidal against 4 microbial strains out of 9 tested, followed by the maceration method, which showed bactericidal activity against 3 microbial strains, and finally the microwave method, which showed bactericidal activity against 2 microbial strains. The Soxhlet extraction method showed the best activity, with a MIC of 125 µg/ml, compared to the other two extraction methods. This better activity could be explained by the presence of

phenolic compounds and alkaloids in the bark, as indicated by the phytochemical screening.On the other hand, a weak activity was observed for the microwave extraction method, which could be explained by the degradation of the molecules of interest due to an increase in power or a prolonged extraction time.The results obtained corroborate with the results of the studies conducted by Fokunang and coworkers. (2013),who also showed that the *_Combretum micranthum_* plant has bactericidal activity against the strain *_Salmonella typhi_*.Additionally, our extracts were tolerant against the strains *_Klebsiella pneumoniae_* and *_Salmonella typhimurium_*, which could be explained by their high pathogenic power compared to the different extracts.

6-Interpretation of antifungal

The antifungal activity was tested on extracts obtained through three extraction methods, namely Soxhlet, maceration, and microwave. For the Soxhlet extract, a moderate activity (125 µg/ml) was observed against the strain *_Candida krusei_ (CK),* and a weak activity (250 µg/ml) against the other 6 strains, with the most significant effects observed against *_Trichophyton mentagrophytes_ (TM)* and *_Epidermophyton floccosum_ (EF)*. Indeed, the extracts were fungicidal against the strains *CK, TM*, and *EF*, with a CMF/MIC ratio ≥ 4.The microwave extract showed a weak activity against the 7 tested strains, with only *Candida albicans_* being sensitive to the extract. Indeed, the extract was fungicidal against this strain, with a MFC/MIC ratio ≤ 4.Regarding the maceration extract, a moderate activity was observed against *_Candida parapsilosis_*, and a weak activity against the strains *CA, CN, TM, MA*, and *EF*. Indeed, the extract was fungicidal against the strains *CA* and *CP*, with a MFC/MIC ratio < 4.None of the extracts had a fungicidal effect against the strains *Cryptococcus neoformans_* and *_Microsporum audouinii_*, which may be attributed to their higher pathogenic power. After analysis, we observed that among the 7 tested strains, the Soxhlet extract exhibited 3 fungicidal activities, the maceration extract exhibited 2, and the microwave extract exhibited only 1. Comparing these results to the previous antibacterial tests, we noticed that the Soxhlet method consistently ranked first, followed by maceration and then microwave. We can deduce that the Soxhlet method is the most suitable for producing active compounds.

7- Interpretation of the antioxidant

The phenolic extracts from *_Combretum micranthum_* obtained through three extraction methods (maceration, microwave, and Soxhlet) have the ability to reduce free radicals (DPPH, ABTS, FRAP). The RSA50 and RC50 values are inversely proportional to the scavenger effect, meaning that lower values reflect a more significant antioxidant effect, expressed in µg/ml.According to H. and coworkers. (2018), the lower the RSA50 value, the more potent the antioxidant potential of the extract. As shown in Table 6, each extract was tested using three methods (DPPH, ABTS, and FRAP), and the results revealed that the Soxhlet extract was more effective in scavenging free radicals, with the lowest concentrations of 20.83, 23.11, and 21.56 µg/ml, respectively, compared to the other methods. Furthermore, these results differ from those of Touoze and coworkers.;(2023), who found that the microwave extract was more effective in stabilizing the unstable DPPH molecules. This discrepancy may be attributed to the difference in the harvesting locations.

8 - Result of the Preliminary Study

8-1-The Influence of Extraction Solvent on Phenolic Compound Content

In view of Figure 3, the highest extraction rate of total polyphenols is recorded by the methanolic extract with a content of 4.70 mg GAE/g of powder, while extraction with ethyl acetate gave a low rate of 1.167 mg GAE/g. Therefore, methanol is the best extraction solvent.

8-2 - The Influence of Microwave Power on Extraction of Polyphenols

We note from our results that the extraction of phenolic compounds was significantly influenced by microwave power. The rates of phenolic compounds increase with increasing extraction power, with a maximum rate of 4.174 mg GAE/g of powder at 40 W. Beyond this power, the rate of these compounds decreases; we record a content of 4.17 mg GAE/g of powder at 50 W.

Figure 4 shows that there is a difference between 10, 20, 30 W compared to 40 W, and no significant difference is observed between 40, 50, 60 W.(**Dahmoune et *al.*,2015)** studied the effect of microwave power on the extraction of total phenolic compounds and found that power significantly increased the contents of total phenolic compounds, which confirms our results. High temperatures influence the solubility of phenolic compounds, increase the diffusion rate with enhanced mass transfer, however, it should be noted that increasing the temperature beyond certain values may favor the risk of thermal degradation and possible decomposition of phenolic compounds (**Li et *al.*,2012**). In this analysis, the optimal interval for microwave power was defined between 30 and 50 W.

8-3 - The Effect of Different Extraction Times

The effect of time on the extraction of total phenolic compounds from Combretum micranthum bark extracts was studied, and the results are summarized in Figure 5.

Figure 5 shows that the extraction of total phenolic compounds occurs continuously. Total phenolic compounds located on the surface of the particle to those located at the heart of the particle reach a maximum value of 7.465 mg GAE/g extract. The maximum value was observed at 50 seconds, followed by a decrease. This decrease may be due to the degradation of total phenolic compounds (Dahmoune et *al*., 2015). It appears from this graph that the maximum time is between 40 and 60 seconds.

8-4 - The Effect of Different Solid-Liquid Ratios on Extraction of Polyphenols

Figure 6 below presents the results observed during the study of the effect of different solid-liquid ratios.

In view of Figure 6, we note that increasing the solid-liquid ratio from 3/20 to 3/100 leads to a better extraction yield, which increases from 1.92 to 5.447 mg GAE/g of powder, respectively. Beyond this maximum value, the rates of phenolic compounds decrease; they pass from 5.447 to 4.387 mg GAE/g of powder, which may be due to the oxidation of total phenolic compounds **(Dahmoune et *al.*,2015).** Based on the results obtained, the solid-liquid ratios 3/80 and 3/120 were chosen as the optimal interval.

9- Results of Optimization

9-1 - Study Domain of Optimization (Refer to Table7)

Preliminary studies allowed us to find the best extraction conditions, which will be used to carry out experimental designs. Using a full factorial design of 8 experiments, we evaluated the influence of time (t), power (p), and ratio (R) on phenolic compounds by microwave-assisted extraction. The experimental responses of the experimental design are presented in the table below.

It appears from Table 8 that time, power, and ratio have an influence on the experimental response (total phenolic compounds) observed. Moreover, it is observed that the contents of total phenolic compounds are between 0.753 - 4.333 mg GAE/g powder. The maximum value of total phenolic compounds (4.333 mg GAE/g powder) is obtained for conditions t = 60 s, P = 50 W, and R = 0.0250 g/mL.

9-2- Experimental and Statistical Modeling_

Statistical analyses showed that the response values fit a first-order polynomial model. This modeling expressed the relationship between the response variable CPT and the test variables. The comparison between experimental and predicted values (Table 13) allowed for the calculation of the determination coefficient R2 for CPT, which was 99.17%. This value, being very close to 100, highlights the agreement between the model and experimental results. **(Hair et *al*., 2014)** consider a model valid if it explains at least 80% of the response variability (adjusted R2). The table shows that the model explains 94.16% of the variance in CPT with a deviation percentage of 5.01%. This analysis indicates that the model is valid.

1-Model Equation_

The results of the model coefficients are presented in the table below.

Table 9: Coefficients and second-order interactions of the model

$Y_{CPT} = -19.08000 + 0.34150\ T + 0.36890\ P + 169.0000\ R - 0.00475\ TP - 1.67000\ TR - 2.48000\ P*R$

2. Analysis of Variance_

An analysis of variance was performed on the obtained results. Table 10 summarizes the results. This analysis allows testing the relevance of variables involved in the studied model and graphically representing the importance of each factor on the studied response. Thus, the linear effect of time and microwave power significantly influences ($p < 0.05$) the yield of total polyphenols from Combretum micranthum bark. It is also worth noting a significant interaction ($P < 0.05$) between time and ratio. The developed model presents a regression coefficient equal to 99.17% (table), indicating good agreement with the experimental phenomenon.

3. Contribution of Different Parameters_

The Pareto diagram informs about the order of significance of each factor. According to the analysis of variance, three factors have a significant effect on yield. However, the Pareto diagram shows only two significant factors (time and power) and a significant time-ratio interaction. For ratio, time-power interaction, and power-ratio interaction, the diagram indicates no significance.

4. Response Surface Analysis_

4-1-Interaction between Irradiation Time and Ratio_

The 3D representation in Figure 8 indicates the influence of time and solid-liquid ratio on the yield of phenolic compounds. From the graph, it can be seen that at a time (40 s) and a volume (80 mL), there is a low yield of total phenolic compounds (1.66 mg GAE/g E). This could be explained by the weak diffusion of the solvent within plant tissues, resulting in poor solubilization of phenolic compounds. Furthermore, when the time is equal to 1 minute and the volume is maintained at 80 mL, an increase in total phenolic compounds is observed, from 1.66 to 3.88 mg GAE/g E. When the volume increases to 120 mL while maintaining the time at 1 minute, the concentration of CPT increases from 3.88 to 4.22 mg GAE/g E. This result is similar to those of (**Li et *al.*, 2012).**

5. Optimal Extraction Conditions for Phenolic Compounds_

This study shows that a complete first-order polynomial model can correctly model the studied phenomenon. It appears that the optimal experimental conditions, i.e., those leading to maximization of polyphenol extraction, are obtained within the experimental domain in the following graph.

These results indicate that obtaining the desired yield (4.2191 mg GAE/g E) requires increasing the extraction time towards the upper limit of the variation domain of this factor (60 seconds) as well as the power (50 watts). These results also affirm that the solid-liquid ratio inversely influences the yield of total phenolic compounds, which reaches its maximum when the solid-liquid ratio is at the lower limit of the variation domain of this factor (3/120 g/mL).

Conclusion

This study aimed to conduct a comparative analysis of three extraction methods for secondary metabolites from the bark of Combretum micranthum, based on quantitative and qualitative analysis, as well as evaluation of the biological activities of the bark extracts.The phytochemical screening results revealed the presence of polyphenols and alkaloids in each extract. Furthermore, the evaluation of the global yield of each extract and the quantification of total polyphenols showed that microwave-assisted extraction yielded the highest extract yield and the highest concentration of total polyphenols.Regarding the evaluation of their biological activity, specifically antimicrobial and antioxidant tests, the Soxhlet extract exhibited the best antimicrobial activity, being bactericidal against four microbial strains, while the maceration method showed the best antioxidant activity, with the lowest RSA50 values for each antioxidant activity method.Moreover, the analysis of variance revealed that the model explains 99.17% of the variance, with an adjusted determination coefficient of 99.17%. The optimal extraction conditions were obtained when the time was set at a high level (60s), the power at a high level (50 Watts), and the ratio at a low level (0.025 g/ml).

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