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Influence of microwave assisted extraction on antioxidant and antiplasmodial activities of *Trichilia roka* extracts

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ABSTRACT

Antiplasmodial and antioxidant activities of extracts derived from *Trichilia roka* (Chiov) (Meliaceae) root bark were determined respectively *in vitro* and using two methods as well as 1,1-diphenyl-2-picrylhydrazyl radical-scavenging and β-carotene-linoleate model systems. The microwave assisted extraction extract was more effective concern antioxydant activity than the antiplasmodial activity compare to conventional mechanical agitation extraction method. Extracts obtained by microwave assisted extraction showed a high total polyphenol content of 126766 µg Equivalent of Gallic acid /g Dry Mater and a total flavonoid content of 789.22 µg Equivalent of Quercetin/g dry mater but a low antiplasmodial activity with and inhibition concentration of 48.386 µg /mL and 23.983 µg/mL for mechanical agitation extract. The evaluation of the antioxidant properties of the two extracts showed that those obtained by microwave assisted extraction shown in the 1,1-diphenyl-2-picrylhydrazyl assay and in the β-carotene bleaching test, the highest antioxidant activity respectively, with an antioxidant activity of 82.12% and with a radical-scavenging activity with inhibition percentage of 88.78%..

Keywords: *Trichilia roka*, Microwave extraction, Antiplasmodial activity, Antioxidant activity.

INTRODUCTION

The use of bioactive compounds like polyphenols, the well known naturally occurring antioxidants, in different commercial sectors such as pharmaceutical, food and chemical industries, signifies the need of the most appropriate and standard method to extract these active components from plant materials. Microwave assisted extraction (MAE) has been used to increase biological activities, to reduce extraction time, extraction solvent, and energy used compared to the conventional method, such as mechanical agitation.

The common source of drugs is natural products and their derivatives in the world public health [1]. More than that, vegetable materials contain many compounds with biological activities which are obtained in form of plants extracts [2]. However, polyphenols are the major plant compounds with antioxidant activity and other several activities associated with healthy properties ascribed to their antioxidant activity and free radical scavenging abilities [3-5]. Polyphenols had also demonstrated to exhibiting a wide range of others biological effects such as antiplasmodial, antioxidant and modulators of various enzyme systems [6]. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in plants for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents [6].

Conventional extraction method like mechanical agitation is highly energy, time, and solvent-consuming [6]. But, Microwave assisted extraction (MAE) is a reliable alternative method of extraction [7]. Compared to conventional solvent extraction methods, Microwave assisted process (MAP) technology offers some combination of the following advantages: Improved products, increased purity of crude extracts, improved stability of marker compounds, possibility to use less toxic solvents, reduced processing costs, increased recovery and purity of marker compounds, very fast extraction rates, reduced energy and solvent usage [6]. As the matrix is also directly heated from the inside to the outside during microwave extraction, it improves the extraction and solubilisation of the root. The major contributory factors to the growing interest in medicinal plant include: rising costs of orthodox medications, low therapeutic index of synthetic compounds and the growing incidence of drug resistance among the pathogens especially in developing countries with very weak economic indices [8]. In the same order, increasing number of ethnobotanic inventories has been established. For example in Cameroun, we can mention some research works [9-13]. However, to the best of our knowledge so far, there is no report about the comparison of antioxidant activity and antiplasmodial activity for *Trichilia roka* extracts obtained by mechanical

agitation and MAE. The aim of this research work is to study the influence of microwave assisted extraction on the antiplasmodial and antioxidant activities of *Trichilia roka* root bark extracts compared to its mechanical agitation extract.

MATERIALS AND METHODS

Plant material

The plant samples were collected in Touboro at about 50 Km from Ngaoundere in December 2008 and identified by Pr Mapongmetsem, a botanist in the Department of Biological Sciences of the University of Ngaoundere. The roots sample of *Trichilia roka* were dried and powdered. An amount of 3.25 Kg was obtained.

Microwave assisted extraction procedure

Extraction of phenolic compounds for different investigation was carry out by using MAE system. The extraction was examined at different extraction times (20 – 80 s) with three other fixed factors: Polarity, microwave power level, and liquid-solid ratio. Aqueous methanol was used as safe and efficient solvent for the extraction of phenolic compounds [14]. In this study MAE procedure was carried out as following: 5 g of dried powder vegetal material was suspended in 100 mL of aqueous methanolic solution (40:60) (v/v) in a 250 mL Teflon extraction vessel. The vessel were placed at the center of the microwave apparatus and heated at different time (20, 30, 40, 50, 60, and 80 s), at 600 W. The vessel was allowed to cool down (1 min at 25°C) then using an immediately filtered system with Whatmann filter paper and funnel to obtained extract. Then, the different extracts were kept at 4°C for further investigations.

Mechanical agitation extraction

Five grams of roots powder of *T. roka* were placed in a 250 mL conical flask, to which 100 mL of 4/6 (v/v) methanol–water solution was added; extracted by mechanical agitation in conical flask, the process time was varied from 2 to 4 hours exactly 120, 150, 180, 210 240 min. Organic solvents were then removed by evaporation under reduced pressure with a Bücchi rotatif evaporator. The extracts and their yield obtained are given in figure 1. The yield of the extraction was calculated as follows:

$$Y\% = M_1/M_2 * 100,$$

Where M_1 (g) and M_2 (g) are respectively the weight extract, the weight of dried powder and Y is the extraction yield.

Determination of total phenolic compounds content

The content of total phenolic compounds (TPC) in *T. roka* extracts was measured using the Folin-Ciocalteau reagent according to the modified method used by Singleton *et al*., 1999 [15]. Briefly, 0; 0.005; 0.01; 0.02; 0.025 mL of the investigated extracts adjusted to 0.5 mL with distilled water in the test tube was shaken for 60 s on the Vortex mixer with 0.5 mL of Folin-Ciocalteu reagent (1/16). After 5 min, 2 mL of Na_2CO_3 (20 g/L) was added and the mixture was shaken once again for 30 s. The mixture was allowed to stand at 40°C for 20 min with intermittent shaking. The absorbance was measured on the Metertech Germany Spectophotometer UV/vis sp 8001 using glass cuve against blank in the first test tube. The TPC was assessed by plotting the gallic acid calibration curve and the results expressed as gallic acid equivalent (GAE) are recalculated as the percent of dry extract.

Determination of total flavonoid compounds content

Total flavonoids were brought up using Dowd method adapted by Arvouet-Grant *et al*. (1994) [16]. Precisely, 1 mL of methanolic solution of 2% aluminum chloride solution $AlCl_3$ was mixed with 1 mL of investigated methanolic extracts (1 mg/mL). The mixture was allowed to stand at room temperature for 15 min with intermittent shaking; the absorbance of the mixture was measured at 415 nm against a blank sample without aluminium chloride using Metertech Germany Spectophotometer UV/vis sp 8001. The content of total flavonoids was calculated and expressed as micrograms of quercetin equivalents (QE) per g of the extract and dried powder.

Quantitative Antioxidant test using DPPH radical-scavenging activity

The antioxidant activity of the plant extracts using the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DDPH) free radical was determined by the method described by Brand [17]. The capturing of free element of a solution of DPPH is observed by the disappearance of the purple colour. That reaction of decolouration, depending on the concentration of the antioxidant substances in the milieu can be followed by spectrophotometry. The protocol of evaluation of these activities is described as follow: The solution of DPPH was prepared by dissolving 4 mg in homogeneous manner in 100 mL of methanol. Extract solution were added to DPPH so as to have 1 mL of solution of 0.1000, 0.0500, 0.0250, 0.0125, 0.0100 mg/mL of concentration in the spectrophotometric cuves. These cuves were introduced in the spectrophotometer and the optical densities read at 517 nm after 30 min of incubation. The negative witness is a solution of DPPH at 10 % in the methanol and the positive witness is the Butylhydroxytoluene (BHT) were submitted to the same analysis and rigorously in the same conditions with the same concentration as the plants fractions. The percentage of inhibition of free DDPH radical (IP %) was calculated by using the formula below:

$$IP\% = (A_{blank}-A_{sample})/A_{blank} * 100,$$

Where IP is inhibition percentage, A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The optical densities obtained helped to draw the graphic according to the inhibition concentrations and the concentration in inhibitors and to deduct the IC_{50} values (table 1)**.**

Quantitative antioxidant test using β-carotene-linoleic acid

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [18]. A stock solution of β-carotene-linoleic acid mixture was prepared by dissolving 5 mg of β-carotene in 10 mL of chloroform and 250 µL of linoleic acid and 2 g of tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of aerated distilled water was added with vigorous shaking to form an emulsion. Aliquots (1.5 mL) of this emulsion were then transferred into different test tubes containing different concentrations (0.1000, 0.0500, 0.0250, 0.0125, 0.0100 mg/mL) of the different extracts. As soon as the emulsion was added to each tube, the zero time absorbance was measured after 5 minutes in a water bath at 490 nm using Metertech Germany Spectophotometer UV/vis sp 8001. Thereafter, the tubes were placed at 50°C in a bath water for a period of 02 h before re-measuring. A blank treatment served as the control for the spectrophotometric readings. The same procedure was repeated with the synthetic antioxidant BHT, as positive control. Antioxidant activity (AOA) was calculated using the following equation:

$$\%AOA = (A_{2H}/A_i)*100;$$

With % AOA: antioxidant activity; A_{2H}: β-carotene content after 2 h of assay and A_i: initial β-carotene content [19].

In vitro antiplasmodial activity

The chloroquine-resistant *Plasmodium falciparum* strain W2 used for this research work were obtained from Rosenthal research group in California. They were maintained in culture in sealed flasks at 37 ◦C

in a 3% O_2, 5% CO_2 and 91% N_2 atmosphere in RPMI 1640, 25 mM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit. Parasites were synchronized in the ring stage by serial treatment with 5% sorbitol (Sigma) and studied at 1% parasitemia [20]. Plant extracts were prepared as 1 mg/mL stock solutions in DMSO, and further diluted as needed for individual experiments, and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 µM artemisinin (Sigma). Cultures were incubated at 37°C, for 48 h. Parasites at the ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. Aliquots (50 µL) of each culture were then added to 5 mL round-bottom polystyrene tubes containing 0.5 mL 0.1% Triton X-100 and 1 nM YOYO nuclear dye (Molecular Probes) in PBS, and parasitemias of treated and control cultures were compared using a Becton-Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes [21]. Data acquisition was performed using CellQuest software. These data were normalized to percent control activity and 50% inhibitory concentrations (IC_{50}) were calculated using Prism 5.0 software (GraphPad) with data fitted by nonlinear regression to the variable slope sigmoidal dose–response formula:

$$Y=100/(1+10^{(\log IC_{50}-x)H}),$$

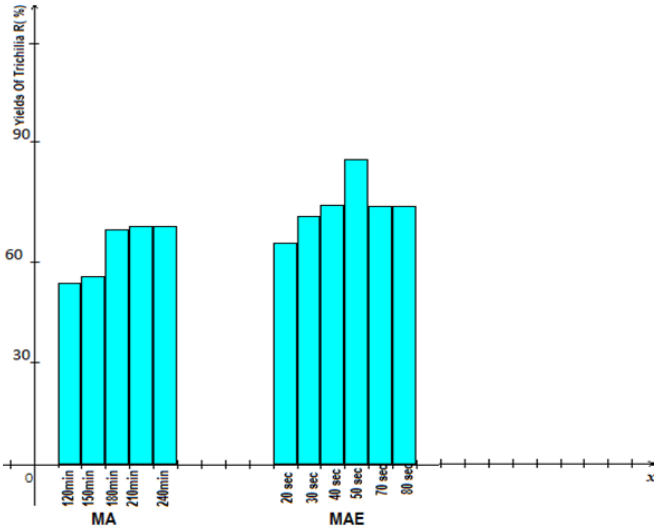
Where H is the hill coefficient or slope factor [22].

RESULTS & DISCUSSION

The comparison of the two kinds of methods

Yield extracts

The results of comparative studies on extraction yields obtained by the two methods are summarised in Fig. 1, which illustrates the *Trichilia roka* extracts obtained by microwave assisted extraction and by mechanical agitation methods as a function of extraction times. The highest extraction yield (89.12%) was achieved by microwave assisted extraction, compared to mechanical agitation method extraction with a lower extraction yield (69.10%). This result can be explained by the fact that Microwave-assisted extraction (MAE) uses microwave energy to extract compounds from plants materials. In the MAE process, the rapid temperature rise, the internal pressure increase, due to the microwave radiation, facilitate permeability and solubilisation processes to leach target constituents of vegetable materials. With the mechanical agitation, the extraction is assigned to the effect of the solvent and the process of agitation that takes place leading an increase of the temperature provoking the leaching of the plant material. For the two methods, increasing of extraction time increased the quantity of extract, until 50 s for MAE and 210 min for mechanical agitation. So that, we observed few extraction time with MAE compared to Mechanical agitation; although there is the risk of the degradation of extracted compounds after that different time (Fig 1). This high microwave extraction yield is mainly due to the fact that microwave energy is delivered efficiently to materials through molecular interaction with the electromagnetic field and offers a rapid transfer of energy to the extraction solvent and raw plant materials [23]. Furthermore, this significant increase in extraction yield, belong also to the higher solvent temperature which has a stronger solubility. In addition, with an increase of irradiation power, boiling point of the mixture would reach more expeditiously, and boiling phenomenon is more intense and the disruptions of root bark sample under microwave irradiation take place. These results concurred with previously published results [24], which showed that applying microwave technique to the extraction of plant secondary metabolites can significantly improve extraction yield compared to conventional extraction method like mechanical agitation. Like other conventional extraction methods, mechanical agitation has the same disadvantages, and is characterized by low efficiency of phenolic extractions [7].

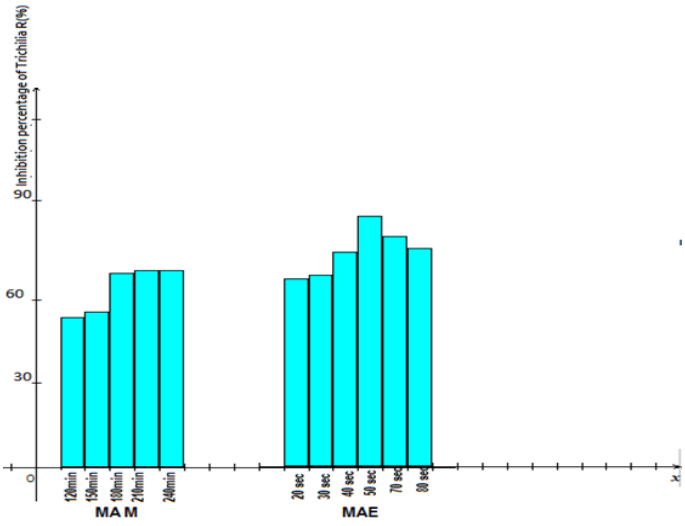


MA: Mechanical agitation, MAE: Microwave assisted extraction

Figure 1: Extraction yields for MAE and mechanical agitation of *Trichilia. roka* roots barks.

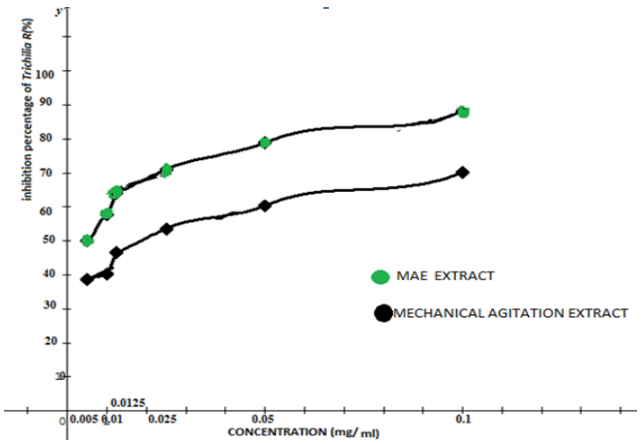
Antioxidant activity

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used and the extraction method [8]. Antioxidant activity was measured for the different extracts obtained at different extraction time by two methods, namely mechanical agitation (MA) and microwave assisted extraction (MAE). The antioxidant activity as a function of extraction method at different times, and as a function of concentration was investigated. Results are shown on Fig 2 and 3. The highest antioxidant capacity of the phenolic compound was achieved by microwave assisted extraction (MAE), (IC_{50} = 0.002 mg/mL) compared to mechanical agitation method (IC_{50} = 0.01 mg/mL). The lower activity of mechanical agitation extract could be resulted from extended extraction time, hence exposure to unfavourable conditions such as light and different molecules interaction.



MA: Mechanical agitation method; MAE: Microwave assisted extraction.

Figure 2: Inhibition percentage *Trichilia roka* extract in function of the extraction method using β carotene and linoleic acid system.



MA: Mechanical agitation method; MAE: Microwave assisted extraction.

Figure 3: Inhibition percentage in function of the concentration for *Trichilia roka* by using β-carotene and linoleic acid system.

Results also showed that the two extracts and BHT at various concentrations prevented the bleaching of β-carotene to different degrees. The β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant, because of oxidation of the couple β-carotene and linoleic acid which generates free radicals [25,26]. *T. roka* extracts prevented discolouration of the couple β-carotene and linoleic acid meaning that the polar extracts, especially rich in water-soluble chemical substances, attributed their antioxidant activity especially to the presence of the phenolic compounds in these extracts. Table 1 below show the values of IC_{50} for different extracts obtained by using the curve of the variation of inhibition percentage in function of concentration (Fig 3). The free radical scavenging activity of MAE extract was the highest one (IC_{50}= 3.3 10^{-3} mg/mL) compare to mechanical agitation extract (IC_{50}= 2 10^{-2} mg/mL). These activities could be related to theirs phenolic constituents, knowing that polyphenols are one of the major compounds with antioxidant activity and other several activities associated with healthy properties ascribed to their antioxidant activity and free radical scavenging abilities [2].

Table 1: IC_{50} value for Free radical scavenging capacities of the *Trichilia roka* extracts measured in DPPH assay.

	Extraction methods									
	MA					MAE				
Conc (µg/mL)	0.1000	0.0500	0.0250	0.0125	0.0100	0.1000	0.0500	0.0250	0.0125	0.0100
IP (%)	66.53	63.53	61.21	55.02	48.45	72.80	70.87	69.45	69.95	68.10
IC_{50} (mg/mL)	2 10^{-2}					3.3 10^{-3}				

MA: Mechanical agitation extract, MAE: Microwave assisted extraction, IP: Inhibition percentage.

Table 2: *In vitro* antiplasmodial test result of *Trichilia* roka extracts.

		***Plasmodium falciparum* W2**	
Extraction method	**Extraction time (s)**	**IC_{50} (µg/mL)**	**SD**
MAE	20	48.386	20.477
	30	>50.000	/
	40	>50.000	/
	50	>50.000	/
	80	>50.000	/
MA	RR	23.983	12.741

MAE: Microwave assisted extraction, MA: Mechanical agitation, RR: extract obtained by mechanical agitation at 180 min; /: Not mentioned (IC_{50} > 50).

In vitro antiplasmodial activity

The antiplasmodial activities of the two extracts are summarised in Table 2. IC_{50} obtained with MAE extract was the highest one (IC_{50}= 48.3 µg/mL) indicated the very weak antiplasmodial activity of MAE extract. To the best of our knowledge, extracts with good *in vivo* activity but lacking *in vitro* antiplasmodial activity is scare [27]. One explanation is that, the microwave with their irradiation phenomenon may have destroyed during extraction, limonoids one of the principal and major constituents responsible of antiplasmodial activities of *Trichilia* species and family Meliaceae [28]. In our previous study, we have observed a decreasing in parasites from the first to the fifth day of treatment, indicates the (MA) methanolic extract of *T. roka* was potentially active against *P. berghei* and this activity grows with concentration for mechanical agitation extract [13]. Based on IC_{50} values, *in vitro* antiplasmodial activities of crude extracts from plant materials can be classified differently; knowing that there are no universal agreed criteria on the classification or definition of *in vitro* antiplasmodial activity [29]. However, some commonly accepted criteria do exist. In our study, the mechanical agitation (MA) extract exhibited a strong activity than microwave assisted extraction which exhibited a moderate activity for the extract obtained at 20 s and no

activity for the extract obtained for more than 20 s according to Kraft classification. Kraft classified crude extracts from Zimbabwean medicinal plants with respect to the *in vitro* antiplasmodial activity based on IC_{50} values as having strong activity (IC_{50} below 30 µg/mL), moderate activity (IC_{50} between 30 and 50 µg/mL) and being inactive for IC_{50} higher than 50 µg/mL [30].

CONCLUSION

Results obtained from this research works revealed that MAE owned the best yield, shortest extraction time, highest antioxidant activity but lower antiplasmodial activity. MAE was proved to be the best choice for the extraction process of *Trichilia roka*. In order to evaluate all parameter that affect the extraction efficiency, further studies need be conducted for kinetic analysis, different microwave power, different methanol polarity, different solid-liquid ratio and different extraction time. MAE procedure was shown to be a promising technique in recovery secondary metabolites with interest biological activities such as antioxidant activity except antiplasmodial activities in *Trichilia roka*.

Conflict of interest: NIL

Source of support: NIL

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