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Microwave assisted extraction of antiplasmodial and antioxidant limonoids from *Trichilia roka* (chiov)

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ABSTRACT

Trichilia roka (Chiov) is a medicinal plant from Meliaceae family. It is recognized in traditional medicine for its innumerable therapeutic properties. Limonoids, the main constituents of the root bark of this plant, is known for its antioxidant and antiplasmodial activities. To obtain an improved yield of these bioactive compounds from *T. roka* and reduce extraction time, solvent and energy required, it is of utmost importance to adopt innovative approaches such as microwave-assisted extraction. Microwave was attempted, as compared with the conventional mechanical agitation method to extract bioactive limonoids and quantify them through colorimetric quantification method using 4(dimethyl amino) benzaldehyde (DMAB). The antiplasmodial activity was evaluated against the intraerythrocytic stages of cultured *Plasmodium falciparum* using a phenotypic approach, and the antioxidant property was evaluated *in vitro* using DPPH radical-scavenging and β-carotene-linoleate model systems respectively. Three limonoids and were isolated from *Trichilia roka* (Chiov) root bark labelled RA, RO and RY. The microwave extraction yields were 115.895 mgRUBE/gDW for limonoids. The isolated compounds exhibited good antioxidant activities than crude extracts with IC_{50} values of 2.59 10^{-3}, 2.26 10^{-3} and 1.79 10^{-3} mg/mL respectively compared to crude extract IC_{50} values of 2. 10^{-2} mg/mL. The *in vivo* antiplasmodial test of the hydromethanolic microwave extracts showed during the five treatment days the decreasing of the parasitaemia for doses 100 mg/kg, 200 mg/kg and 500 mg/kg with inhibition percentages of 82.75, 84.84 and 87.8 respectively.

Keywords: *Trichilia roka*, Limonoids, Microwave extraction, antiplasmodial activity, antioxidant activity, DMAB assay.

INTRODUCTION

Trichilia roka. Chiov. (Meliaceae) is a medium sized evergreen tree of the Meliaceae family up to 21 cm high with large trunk up to 15 cm in diameter, low drooping branches, a crown with evergreen leaves clustered in tufts. Flowers appear as short, dense terminal racemes of greenish color. The fruits are red globose berries when ripe, then brown. Found in central Africa, Sudan and Uganda, *T. roka* is largely distributed in the savannah area of the Adamawa region of Cameroon and it is named 'Sulafinzan' in the language of Bambara [1]. This family, also distributed in tropical and subtropical regions, includes more than 50 genera with about 1400 species [2]. Of the various secondary metabolites isolated from different parts of Meliaceae plants, tetracyclic terpenes are the most abundant; they are also found but in scarce manner, in the Rutaceae and Cneoraceae families [3]. Limonoids are described as exhibiting a range of biological activities, with insecticidal, antifungal, antimalarial, antibacterial, antiviral, and anticancer properties [4]. The tropical genus *Trichilia* is one of the 50 genera in the Meliaceae family [5]. Screening programs focused on plants belonging to the Meliaceae family have identied the genus *Trichilia* as a plant source with potential for the development of new bioactive compounds, in addition, the compounds derived from terpenes pathway were more significant, corresponding to about 87.7% of isolated and identified compounds from various *Trichilia* species. Among the different terpenoid skeletons of this kind, limonoids were meanly reported, appearing a total of 33.9% of compounds isolated from several *Trichilia* species [6]. These compounds exhibited different biological activities such as anti-inflammatory, cytotoxic, hepatoprotective, antiplasmodial, antioxidant. Therefore, we started a phytochemical investigation of plant belonging to Meliaceae family in order to isolate these molecules. *Trichilia* comprises 90 species distributed in tropical America, continental Africa and madagascar, and it is widely use as antimalarial, and antipyretics in traditional medecine [7]. Countless researchers have isolated limonoids with interesting biological activities from different species of the genus *Trichilia* but their bioactivity remains unexplored. More than that *T. roka* is used for medicinal purposes against various diseases such as cold, bronchial inflammation, hepatic disorders, as antipyretic and antimalarial agent. Previous Sanogo studies showed the hepatoprotective activities of *T. roka* against CCl4-induced liver injury *in vivo* [8]. Limonoids or polyoxygenated terpenoids biosynthetically related to quassinoids whose antiplasmodial, antifeedant, antimicrobial and cytotoxic activities have been demonstrated. More, gedunin, nimbinin, nimbolide, dihydrogedunin, azadirachtin, trichilin A and trichilin B possess high

antiplasmodial properties [9]. The biological activities of limonoids made them attractive targets for both biomedical and synthetic purposes (Fig.1) [6]. That why many limonoids have been screened for *in vitro* antiplasmodial and antioxidant activity and by *in vivo* antiplasmodial test. People used plants for their nutritional purpose but after the discovery of medicinal properties, this natural flora became a useful source of diseases cure and health improvement across various human societies. However, the emergence of multidrug-resistance strains has raised an urgent need to search for new drugs. Otherwise, plants have usually been used in many tropical and subtropical countries to treat malaria and other symptoms associated with malaria [10]. So, many plants like *Artemisia annua, Cinchona Trichila emetica* and many others, have yielded antimalarial drugs that are highly effective against multidrug-resistant *P. falciparum* parasites. These results have encouraged other investigators to screen plant extracts, by isolating bioactive compounds and some of these isolated compounds have shown high antioxydant and antimalarial activities. Because of that antimalarial drugs resistance, new drugs are urgently needed and thus, efforts have been geared towards finding new drug leads from natural resources. Besides this, the harmful side effects of synthetic drugs make the population adopt more and more an attitude of "return to nature" and, consequently, they are now referred to as "alternative medicine". It is known that many antioxidant substances have anticancer or anticarcinogenic properties. It becomes obvious that plant kingdom becomes a rich source of antioxidant compounds, and potentially antiplasmodial limonoids.

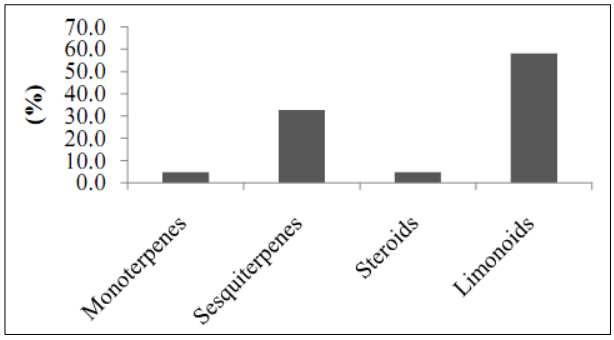

Figure 1: Chemical compounds from the roots of *Trichilia***.** (Vieira *et al* 2014)

Conventional solvent extraction (CSE) generally used for extraction of bioactive molecules from natural sources involve the use of organic solvents such as acetone, methanol, ethanol, ethyl acetate, and hexane. More than solvent consuming, this method is highly energy and time-consuming and often have low extraction efficiencies. Above all, Conventional methods are not environmentally friendly because of the large volumes of organic solvents used which pollute the environment. All these drawbacks have been overcome by the use of an uncommon developed technique for solid-liquid extraction with positive environmental impact and microwave as the source energy called Microwave assisted extraction (MAE). This method was reported to be an efficient extraction method in term of selectivity, yield, speed and it is an environmental protecting method by using less solvent [11]. But the efficiencies of conventional and non-conventional extraction methods mostly depend on the critical input parameters; understanding the nature of plant matrix, chemistry of bioactive compounds and scientific expertise.

However, to the best of our knowledge, there is no report about the microwave extraction and isolation of pure bioactive limonoids from *Trichilia roka* extracts. So the present study, was undertaken to provide on the one hand a comprehensive uncommon technique for extraction named microwave assisted extraction, to isolate active pure compounds from microwave assisted extraction extracts, and to evaluate the antiplasmodial and antioxydant activity of *Trichilia roka* microwave assisted extraction extracts and precisely root bark extracts.

METHODOLOGY

Plant material

Root and stem bark sample were collected in a suburb of the city of Ngaoundere in the month of November 2018. These plant samples were identified and authenticated by Professor Mapongmetsem Pierre Marie, a botanist and agroforester in the faculty of Sciences of the University of Ngaoundere. These samples were air-dried and powdered. Amount of 4 kg and 3.50 kg was obtained for root and stem barks respectively.

Microwave assisted extraction procedure

This technique was carried out according to the same procedure as in our previous work in 2015. Indeed, during different times (10, 20, 30, 40, 50, 60, and 80 s) and a fixe irradiation power 600 W. After microwave heating, the mixture in the extraction vessel is cooled down at room temperature and filtered thus different microwave assisted extraction extracts were obtained. After this step, these extracts of the different ten series were kept at 4°C for future uses.

Conventional solvent extraction

Five grams of air-dried powdered *T. roka* root bark were placed in a 250 mL conical flask, to which 100 mL of 70% (v/v) methanol–water solution was added. The flask with blends was extracted by mechanical agitation at room temperature (25 C). The process time was varied from 120 to 240 min. The solvents or organic layer were then removed by using a Bücchi brand rotary evaporator under reduced pressure. The different extraction yields were calculated as a percentage using the extract weight and the air-dried powder weight.

Pure compounds isolation procedure

Several separation and purification methods such as thin layer chromatography, column chromatography, preparative thin layer chromatography, and recrystallization have been used to isolate the pure compounds from the extracts obtained by microwave assisted extraction of *T roka* root bark. A thin layer chromatography analysis of MAE fractions showed that the seven extracts were qualitatively identical. They were combined and dried using a rotavapor apparatus. A greenish crystalline extract (150 g) was obtained and chromatographed over silica gel (230-400mesh) columns, using a gradient of n-hexane, n-hexane-EtOAc (1:1), EtOAc, $CHCl_3$-MeOH (1:1), EtOAc-MeOH (1:1) and MeOH. This elution yielded six fractions, F_1 (40 g), F_2 (15 g), F_3 (20 g), F_4 (24 g), F_5 (10 g) and F_6 (16g). After the fractionation process, fractions were submitted to a thin layer chromatography (TLC). F_1 and F_4 were those containing more isolable compounds. Fractions two and three were a complex mixtures of very high polarity compounds; F_5 and F_6 were similar and combined for further work. F_1 (40 g) was subjected to column chromatography over silica gel and eluting with only n-hexane-EtOAc and EtOAc to yield three compounds named F'_1 (3 g), F'_2 (7 g), and F'_3 (16 g). After a TLC analysis, the two first fractions exhibited different compounds with the same retarder factor; the last fraction

(16 g) yielded two reddish-orange compounds labelled Ra (8 mg); and Ro (10 mg). F_4 (24 g) was subjected to a column chromatography over silica gel and eluted with $CHCl_3$, $CHCl_3$-MeOH (3:1) to yield three compounds labelled F'$_{41}$ (13 mg), F'$_{42}$ (27 mg), and F'$_{43}$ (36 mg). The two last fractions shown a mixture of different compounds; the first fraction (13 mg) yield one red-orange compound labelled Ry (5 mg) after recrystallization.

So, three pure limonoids according to qualitative test were isolated, close to a powder, they came in the form of very thin reddish-orange granules. The masses of (8 mg); (10 mg) and (5 mg); were obtained and labelled RA, RO and RY respectively. Further studies, precisely spectral analyzes will be conducted for the compounds structure elucidation by spectroscopic methods.

Preparation of 4-dimethylaminobenzaldehyde (DMAB) indicator reagent

The Ehrlich's reagent was prepared based on the methods of Vaks, Lifschitz and Abbassi with some modification. Perchloric acid (70%) 240 mL and glacial acetic acid 300 mL were combined to prepare a stock acid solution. The DMAB indicator reagent was freshly prepared by dissolving 1.1g in 30 mL of the stock acid solution [12, 13].

Determination of total limonoids compounds (TLC) content

The total limonoids content (TLC) in *T. roka* extracts was measured by using chloroform extraction. 1 mL of microwave extract and 2 mL of chloroform was vortexes in order to remove polar substances. The mixture was vigorously mixed for 2 min using a shaker. After phase separation, 1 mL of the chloroform phase was transferred into another test tube and 1.5 mL of Burnham reagent, which consisted of 4-dimethylaminobenzaldehyde (0.1 g), glacial acetic acid (3 mL) and perchloric acid (2.4 mL), was added. This mixture was kept at ambient temperature for 30 min in order to gain a maximum of red colour (Fig 2). The absorbance of the upper phase was reader at 503 nm. This absorbance was measured on UV/visible spectrophotometer (Spectroquant® Pharo 100M) using glass cuvettes against blank in the number one test tube (the mixture instead test samples).TLC analyses were assessed by plotting the Rubenscin J (Fig. 3), a pure limonoid isolated by Tsamo [14] from *Trichilia rubescens* calibration curve by introducing 0; 0.02; 0.04; 0.06; 0.08 mL in different test tube with Erlich reagent 0.1g glacial acetic acid 3 mL and perchloric acid 2.4 mL. The results is expressed in Rubenscin equivalent per gram of dry matter. Knowing that the colorimetric quantification is based on the formation of red to orange colored derivatives resulting from the treatment of limonin, limonin glycoside, or a plant material extract with 4-(dimethylamino)benzaldehyde (DMAB) in the presence of perchloric and acetic acids. Absorbance maxima for the limonin and limonin glycosides derivatives were found to be 470 and 503 nm, respectively [15].

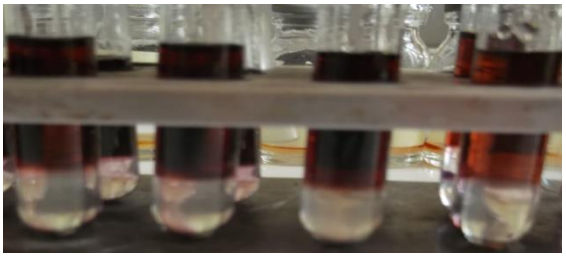

Figure 2: Specific limonoids test by colorimetric method (DMAB) of Ra, Ro and Ry, and Rubenscin J

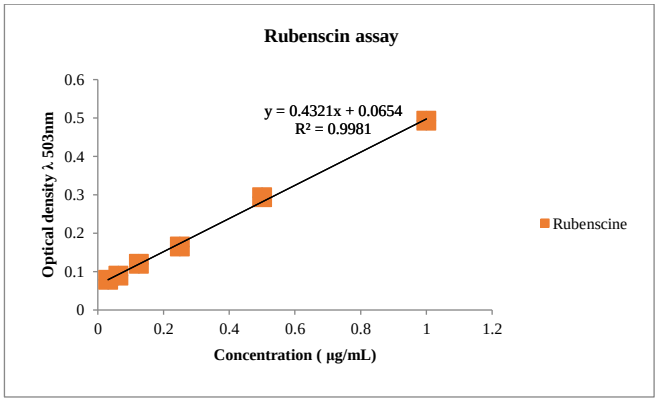

Figure 3: Calibration line of TL

Quantitative antioxidant activities

In order to compare the antioxidant activities of microwave assisted extraction extracts and those of the pure compounds or pure limonoids isolated from these extracts, the experiments were carried out under the same conditions as in our previous work, [22] and by identical antioxidant methods. These methods were radical scavenging activity using, 2, 2-diphenyl-1-picrylhydrazil (DPPḢ) free radical as described by Brand [16], and oxidative degradation of β-Carotene. β-Carotene ability to interact with free radicals results from their conjugated double bond system. This high unsaturated structure render them open to oxidation reactions. In addition, measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [17, 18, 19]. These two methods are used for a rapid and reliable measurement of the *in vitro* antioxidant. That is why they are located at 90 and 30 on a use scale of 100, respectively for DPPH method and for the oxidative degradation of β-Carotene [20]. The obtained optical densities (ODs) were used to draw the graph and IC_{50} were deducted.

***In vivo* antiplasmodial microwave assisted extraction extract activity**

Samples having been extracted with a different extraction method from that of our previous work could significantly influence the results of the *in vivo* antiplasmodial evaluation. This evaluation was carried out according to the same procedure as in our previous work in 2013. Indeed, the *in vivo* antiplasmodial test was done according to the 4-day suppressive test of Peters recommended by OMS with a slide modifications [21]. The main step were the strain conditioning and the rodent's treatment. The treatment microwave assisted extract doses, chosen into account the pharmacokinetics of the drug and the body weight of humans, 500, 250 and 100 mg /kg; were daily administrated intraperitoneally (i.p). Slides were inspected under 100 X magnification optical microscope and parasitaemia was determined by counting 10 fields each containing 100 red blood cells per field and the parasite densities values was carried out by the OMS (1982) formula below:

D = 50* number of parasites/number of read scapes for the full bloated parasitary densities.

Statistical analysis

Differences between control and test sample results obtained for different assays were analysed by the Student t-test. So, values are given as means ± S.D and statistical signifance between treatments was set at $P < 0.05$.

RESULTS AND DISCUSSION

Microwave assisted extraction and conventional solvent extraction yields

The results on extraction yields of microwave assisted extraction methods as a function of extraction times of *Trichilia roka* extracts, is summarised in figure 4. We can noticed that, the highest extraction yield (71.32%) was achieved by microwave assisted extraction, knowing that, Microwave-assisted extraction (MAE) is a method that uses microwave energy to extract compounds from plants materials [22]. This 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]. This significant increase in extraction yield, belong to the higher temperature solvent which has a stronger solubility. In addition to this, 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, 25]. Which have shown that applying microwave technique to the extraction of secondary metabolites from plants can significantly improve extraction table (1).

Table 1: Extraction yields of microwave assisted extraction of *Trichilia roka* root bark extracts for Total Limonoids (TL), Scavenging DPPH activity, and antioxidant activity using β-carotene degradation.

Run	DPPH (%)	AOA (%)	TL (mgRUBE/gDW)
1	20.6736	81.0027	29.588
2	18.0023	80.8437	49.9921
3	49.9419	81.3977	34.1269
4	67.1312	84.3412	48.7241
5	62.485482	67.5583	65.5546
6	47.3868	72.2958	65.7852
7	51.6840883	75.0628	50.0361
8	76.3066202	78.4541	48.7241
9	72.8222997	80.1299	49.1523
10	40.0697	75.3325	70.2764
11	68.524971	74.7069	90.8032
12	67.7119628	69.398	108.515
13	69.338	83.622	93.6056
14	56.504065	81.8254	101.329
15	34.1980488	82.8609	113.702
16	43.554	83.4182	115.893
17	36.79432	66.5412	88.823
18	39.1405	65.6397	95.4227
19	41.9279907	78.8438	78.581
20	42.6893	83.1667	96.2185
21	42.8571	75.8966	51.6829
22	58.686321	76.2279	83.6532
23	90.1278	90.4952	35.1597
24	84.0883	89.528	100.791
25	77.8362369	81.6092	89.5324
26	77.4878049	79.4765	89.225

YTL (mgRUBE/gDW): Recovery of total limonoids
YDPPH (%): DPPH scavenging activity yields
YAOA (%): Antioxidant yields using the *β*-carotene bleaching test

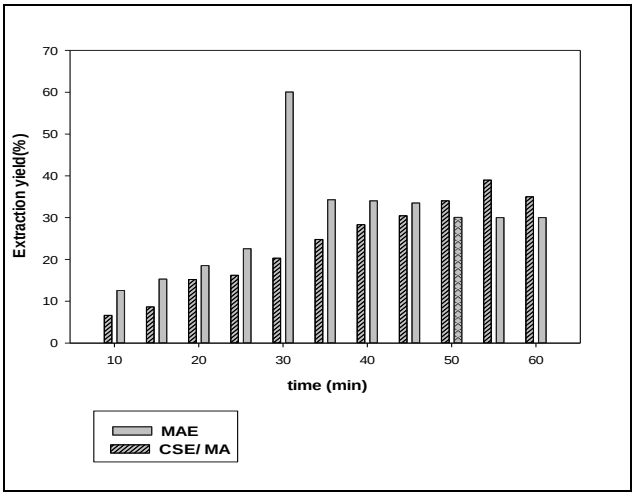

Figure 4: Different yields for MAE and mechanical agitation extracts from *Trichilia roka*

Antioxidant activity of limonoids

The three limonoids (RA, RO and RY) isolated from *T. roka* root bark exhibited higher radical-scavenging activity than the crude extracts, with IC_{50} values of 2.59 10^{-3}, 2.26 10^{-3}mg/mL and 1.79 10^{-3} (Fig.6 to Fig.9). The low antioxidant activity of crude extracts may be explained by the eventual antagonist effect between compounds inside the extract that could decrease the activities, in addition, the presence of some non-polar compounds could also decrease the scavenging activity. On the other hand, limonoids structures are not optimised to provide direct radical-scavenging activity as they lack aromatic and phenolic structures [26]. These results are similar to those of Sz-jie wu [27], where *Citrus grandis* Osbeck Shihtouyu (Shihtouyu) displayed the highest 2, 2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) content (9.3 mg trolox equivalent antioxidant content/g), indicating its good free radical-scavenging activity. *C. grandis* Osbeck Taipeiyu (Taipeiyu) showed the highest 1, 1-diphenyl-2-picrylhydrazyl content and this compound too possesses good radical-scavenging activity. More than that a preliminary phytochemical analysis of *Trichilia emetica* extracts showed a high polyphenol content in the aqueous extract and the presence of limonoids in the ethyl ether fraction. Microwave extract is more effective than conventional solvent extract (table 2). This activity may be a result of the presence of polyphenols and limonoids in *Trichilia roka* extracts. It was found that IC_{50} values of Ra, Ro, and Ry are less than that BHT one (IC_{50} value of 3.4 10^{-4}µg/mL). And using β carotene and linoleic acid system, microwave assisted extract (IC50) is less than conventional solvent extraction one. (Fig.6).

Table 2: IC_{50} value for antioxidant capacity of the *T. roka* extracts using DPPH assay.

Method	[conc] (µg/ml)		0.1000	0.0500	0.0250	0.0125	0.0100	IC_{50} (mg/ml)
CSE	MeOH/H_2O (4:6)	IP%	66.53	63.53	61.21	55.02	48.45	2 10^{-2}
MAE	MeOH/H_2O (4:6)	IP%	72.80	78.87	69.45	69.10	75.10	4.2 10^{-4}

CSE: Conventional solvent extraction
MAE: Microwave assisted extraction
IP; Inhibition percentage

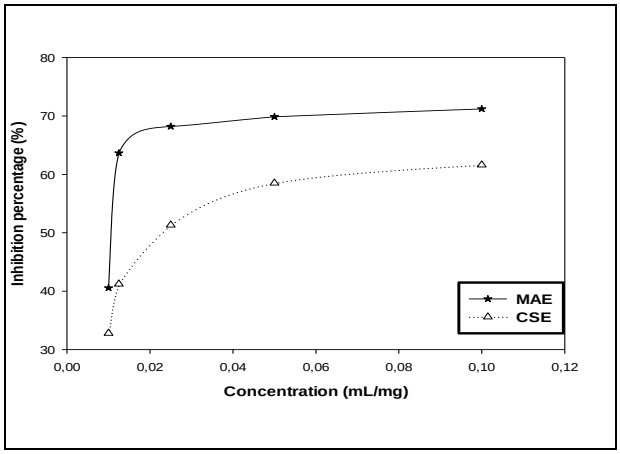

Figure 5: Inhibition percentage of *T. roka* extract in function of the concentration by oxidative degradation of β carotene for the two methods investigated.

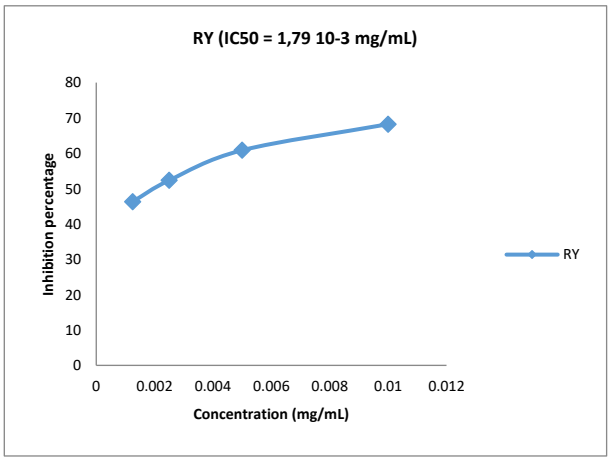

Figure 6: Inhibition percentage of compound RY in function of the concentration.

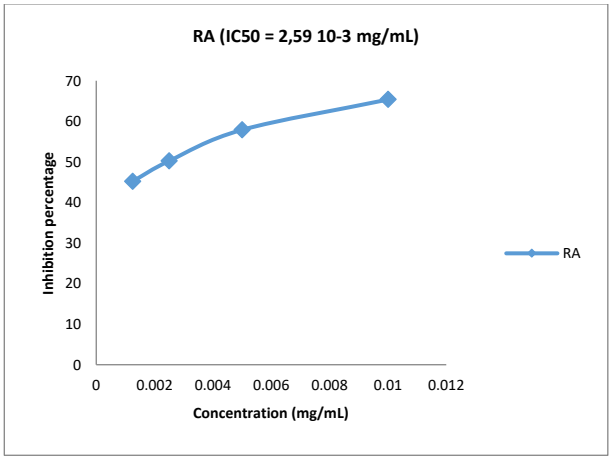

Figure 7: Inhibition percentage of compound RA in function of the concentration

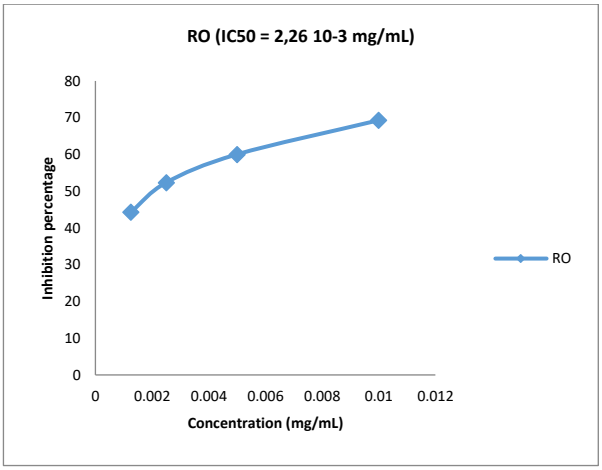

Figure 8: Inhibition percentage of compound RO in function of the concentration.

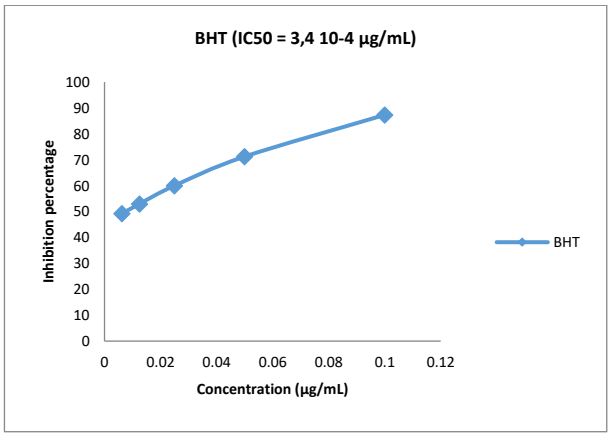

Figure 9: Inhibition percentage of compound BHT in function of the concentration.

Antiplasmodial activity

In this work *in vivo* antiplasmodial investigation of microwave assisted extraction extract shown a good *in vivo* antiplasmodial activity. The hydromethanolic (MeOH/H_2O 40:60) microwave extract of doses 100, 200, 500 mg/kg exhibited a decrease in the parasitaemia from 36.6 to 25.2; from 23.6 to 10.1, from 31.3 to 4.0 on day1 to day 5 post infection (table 3) with a LD50 of 1.157mg/kg (table 4). These extracts allowed a significant reduction of parasitaemia compared to the negative control but were less effective than a Cinchona extract. In our previous work, we observed a week *in vitro* antiplasmodial with IC_{50} of 48.386 mg/ml for methanol extract [22]. The good *in vivo* activity of hydromethanolic (MeOH/H_2O 40:60) could be explained by the need of a metabolization of polar molecules to become effective, an effect on a parasite stage other than the erythrocytic one tested *in vitro* or the different mode of extraction. A similar result were obtained by Bah, reported moderate antiplasmodial activity for the dichloromethane leaf extract of *T. emetica* (IC_{50} =12 mg/ml) [28].

The antiplasmodial activities of the extracts are summarised in Table 3. To the limit of our knowledge, extracts with a significant *in vivo* activity but lacking *in vitro* activity are not usual [29]. One logical explanation may be that, the irradiation microwave phenomena can destroy limonoids in the methanolic extract, which is the main constituents of *Trichilia* gender and *Meliacea*e family responsible for antiplasmodial activities [30]. From the first to the fifth day of treatment, *in vivo* antiplasmodial results revealed a decreasing rate in parasites charge, which indicates that *T. roka* hydromethanolic (MeOH/H_2O 40:60) extract, was potentially active against *P. berghei* and this activity grows with concentration. The good activity of the hydromethanolic (MeOH/H_2O 40:60) extract as shown in table 3 may result from the presence of high polar molecules which exhibit biochemical functions responsible of trophozoids destruction due to the nature of extraction solvent. Further studies would be carried out to investigate the antiplasmodial activity of isolated limonoids compounds RA, RO and RY, the influence of the microwave assisted extraction time, others extraction parameters on antiplasmodial activity of *T. roka* extract and review this acute toxicity shown by the LD50.

Table 3: *In vivo antiplasmodial* test result of *T. roka* root bark microwave assisted extracts.

MAE	Doses	Days of traitement	Parasitemia ± SEM	Parasites densities ± SEM
Hydromethanolic extract	100mg/kg	J_1	36.6 ± 4.3^c	185.2 ± 11.7
		J_2	29.5 ± 2.0^b	146.3 ± 2.3
		J_3	28.3 ± 1.9^a	142.4 ± 1.1
		J_4	27.0 ± 1.7^a	134.7 ± 3.2
		J_5	25.2 ± 1.1^a	125.6 ± 1.4
	250mg/kg	J_1	23.6 ± 1.2^d	116.2 ± 4.6
		J_2	15.6 ± 0.8^c	75.1 ± 2.2
		J_3	13.0 ± 0.7^b	65.4 ± 1.9
		J_4	11.8 ± 0.2^a	56.1 ± 1.1
		J_5	10.1 ± 0.1^a	50.2 ± 0.7
	500mg/kg	J_1	31.3 ± 1.9^d	154.2 ± 13.6
		J_2	11.2 ± 1.1^c	56.5 ± 4.5
		J_3	9.6 ± 1.0^b	44.7 ± 4.1
		J_4	5.3 ± 0.2^a	26.1 ± 0.4
		J_5	4.0 ± 0.4^a	19.8 ± 2.0

Table 4: Inhibition percentage of *in vivo* antiplasmodial activity of microwave assisted extraction extracts *of T. roka* root bark.

Extraction method	Doses (mg/kg)	Inhibition percentage	LD50
Hydromethanolic extract (MAE)	100	82.75	1.157 mg/kg
	250	84.84	
	500	87.8	

CONCLUSION

Microwave-assisted extraction was determined to be an effective method for extracting bioactive compounds from *T. roka* root bark. Values for total limonoids content were affected by the extraction method; the three limonoids RA, RO and RY exhibited potent antioxidant activity, their chemical structures need to elucidate as well as their antiplasmodial activities should be assessed. Our results revealed that MAE is the most suitable extraction method of the dried *T. roka* root bark compare to conventional solvent extraction. It is promoted high yield of the crude extract, the highest contents of total limonoids major active compounds, and the most potent antioxidant activity and then it must be selected for the using of optimization of extraction parameter and kinetics analysis for the further studies. In addition, the microwave assisted extraction IC_{50} is 2 times higher compared to the traditional extraction technique one, suggesting the efficiency of the MAE. The decreasing of parasitaemia from the evaluation of *in vivo antiplasmodial* test investigated in this work also shown that oral administration of microwave assisted extraction hydromethanolic extract of *T. roka* root bark, was active on *Plasmodium berghei*. Then supports the validity of its traditional usage for malaria treatment in folk medicine in Mali. It is also shown by our results that the microwave extract of *T. roka* can be adopted as an effective and efficient safe antioxidant and antiplasmodial source, despite the fact that the antioxidant activities of hydromethanolic extract fraction of *T. roka* root bark extract were lower than BHT and cinchona extract as positive controls. If the pharmacological properties of *T roka* turn out to be favourable, they could become realistic alternatives to modern drugs which are unaffordable or inaccessible to the poor African populations.

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