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Antiplasmodial activity of *Vernonia cinerea* Less (Asteraceae), a plant used in traditional medicine in Burkina Faso to treat malaria

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Research and development of new antiplasmodial molecules in plant is a very important way for the development of new anti-malarial drugs. In this study, *Vernonia cinerea* Less (Asteraceae) was selected for its promising antiplasmodial activity because it is traditionally used in Burkina Faso to treat malaria. The aim of this study was to investigate the antiplasmodial activity of this whole plant. Five crude extracts of *V. cinerea* Less were prepared from the solvents of increasing polarity (CH_2Cl_2, CH_3OH, CH_3OH/H_2O (1/1), H_2O and alkaloids extracts). The method of Ciulei (1982) and thin layer chromatography were used for chemical characterization. The p-LDH technique was used *in vitro*. Extracts were evaluated *in vitro* for efficacy against the *Plasmodium falciparum* strain K1, which is resistant to chloroquine, and 3D7, which is sensitive to chloroquine. The crude extracts of alkaloids showed the IC_{50}=4.25 µg/ml with the strains 3D7 and IC_{50}=2.56 µg/ml with the K1 strains. The CH_2Cl_2 extracts showed the IC_{50}= 8.42 µg/ml and IC_{50}=5.85 µg/ml on strains 3D7 and K1, respectively. The CH_3OH extracts showed the IC_{50}=21.08 µg/ml, CH_3OH/H_2O extracts gave 41.56 µg/ml and H_2O extracts gave 37.17 µg/ml on strains of *P. falciparum* K1. The present study highlighted the very promising antiplasmodial activity of *V. cinerea* Less. The most antiplasmodial activity of this plant extracts merit further study about its *in vivo* antiplasmodial activity in *Plasmodium berghei* infected mice.

Key words: *Vernonia cinerea* Less, alkaloids, triterpenes, antiplasmodial activity, *Plasmodium falciparum.*

INTRODUCTION

Malaria is a potential fatal parasitic disease that remains a public health concern in tropical countries, thus also in Burkina Faso. Globally, 198 million cases of malaria are registered per year, causing about 438 000 deaths (WHO, 2015). The burden is particularly heavy in Africa where 90% of all fatal cases occur, of which 78% occurs in children under the age of five (WHO, 2014). Efforts for malaria eradication have been made from 2000 to 2013

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(WHO, 2014), but this is hampered by the resistance of *Plasmodium falciparum* to antimalarial drugs available (Dondorp et al., 2012). The history of malaria has shown that plants are a source of new molecules. This is the case of quinine that has been isolated from *Chincona* and artemisinin that has been isolated from *Artemisia annua* (Batista et al., 2009; Bero and Joëlle, 2011; Bero et al., 2009; Kaur et al., 2009; Nogueira and Lopes, 2011; Phillipson and Wright, 1991). Over 80% of the world's population use medicinal plants for healing (WHO, 2008, 2010). In Burkina Faso, the majority of the population use medicinal plants as the first therapeutic means (Bero et al., 2009; Traoré et al., 2009) in order to contribute to find new antimalarial molecules having a wide margin of safety and efficiency. In traditional medicine, *Vernonia cinerea Less* (Asteraceae) has many therapeutic uses. Its vernacular name is "little ironweed (USA)" and the whole plant is used in therapeutic. It is used to treat malaria fever, vomiting, inflammation, infections, diuresis, cancer, abortion and gastrointestinal (Jain and Puri, 1984). The decoction is used to treat cardiac pathologies, wounds, colic and diarrhea (Rivière et al., 2005). In Burkina Faso, the plant is recommended in the care of malaria and for the care of dysentery and wounds in therapeutic use. The dosage used is usually a decoction of 50 g/l (Http://www.jardinsdumonde.org). In the face of resistance to artemisinin which is the core molecule of ACT, which extracted from *A. annua* (Asteracea), the priority is to research of new active molecules against emerging resistant strains. It was in this context that we chose *V. cinerea* Less which is a plant of the Asteracea family. The aim of the study was to evaluate the antimalarial activity of extracts from *V. cinerea* Less (Asteraceae) that is used in traditional medicine in Burkina Faso to treat malaria.

MATERIALS AND METHODS

Plant collection

The plant was collected in the Comoé region (West Burkina Faso), GPS 10°38'N, 4°45'W in August 2010 and authenticated by a botanist M. Madou Ouedraogo from the Comoé Regional Forestry Department. After collection, a voucher specimen of plant was deposited in the herbarium of Centre National de Recherche et de Formation sur le Paludisme (CNRFP) in Burkina Faso (Ouagadougou). Then the plant was sprayed and the raw material obtained was sent to the laboratory of pharmacognosy for preparation of crude extracts.

Preparation of plant extracts

Five (5) types of extracts were prepared from the plant powder. We obtained three organic extracts, one aqueous with water and one crude alkaloid. Crude organic extracts were prepared by maceration for 16 h successively with dichloromethane (CH_2Cl_2), methanol (CH_3OH) and water-methanol (CH_3OH/H_2O) solvents. Plant powder (10 g) was used for these organic extraction methods with 100 ml of each solvent. CH_2Cl_2 extract was air dried at room temperature. CH_3OH and CH_3OH/H_2O extracts were freeze-dried with lyophilisator (Brand) after total evaporation of solvents. Aqueous extracts were prepared by boiling 10 g of plant powder in 100 ml of purified water for 30 min. After cooling, solutions were filtered on cotton wool and freeze-dried. Crude alkaloid extracts were obtained by alkalization with 28% NH_4OH to pH9 of the plant powder and extraction with CH_2Cl_2 for 24 h. Plant powder (10 g) was used by applying the classical alkaloids extraction method (Sanon et al., 2003). After 16 h of maceration with ammoniac and CH_2Cl_2, a percolation was made with CH_2Cl_2 solvent. Then 100 ml of CH_2Cl_2 layer was concentrated under vacuum and then extracted with a 3% solution of H_2SO_4 to pH3. The aqueous acid solution was alkalinized again with NH_4OH and extracted with CH_2Cl_2, and a crude alkaloid extracts was obtained by concentration. The yields were calculated using the following:

$$\text{Extracts Yields} = \frac{\text{Mass of the crude extract obtained}}{\text{Test portion of the powder} - \text{plant}} \text{X } 100 \quad (1)$$

Characterization of chemical groups

Phytochemical screening

For characterizing the major chemical groups, non-hydrolyzed extracts and hydrolyzed extracts were prepared. 1 g of each lyophilizate was weighed and mixed with 100 ml of distilled water then decanted into a bottle to get non-hydrolyzed extracts. To obtain hydrolyzed extracts, 25 ml of non-hydrolyzed extracts were removed and mixed with 15 ml of 10% HCl, this was heated under reflux for 30 min. After cooling, the mixture was transferred to a separating funnel. The liquid-liquid partition was done by the addition of 3×10 ml of dichloromethane. The organic phase was recovered and filtered and then stored in vials.

Phytochemical screening of plant extracts was made according to Ciulei method (1982). Chemical groups were identified by liquid medium characterization tests of the extract. Triterpenes and sterols were identified with Liebermann-Büchard test. Tannins presence has been highlighted by the reaction of $FeCl_3$ 1% test tubes. Saponins were identified with the observation of persistent foam column. Coumarins were detected with NH_4OH 10%, UV (366 nm). Emodols and anthracenosids were identified with the Bornträger test. Carotenoids were detected with the H_2SO_4.

Chromatographic analysis thin layer (TLC)

Five microliters of each of the dichloromethane extracts, non-hydrolyzed extracts and hydrolyzed extracts were deposited on chromatography plates (silica gel G60, Merck). The plates were dried in ambient air and placed in migration vats covered before hand containing appropriate solvent systems. The distance covered of the eluent (solvent front) is predefined at 8 cm from the extracts of the deposition line. At the end of migration, the plates were removed and oven dried, and then the UV (254 or 366 nm) was read and after visualized with a reagent specific to the desired chemical groups. Alkaloids were identified with migration solvent toluene-ethyl acetate-diethylamine (17.5: 5: 2.5) and revealed with Draggendorf test. Triterpenes and sterols were identified with migration solvent n-hexane-ethyl acetate-toluene (6: 2: 4) and revealed with sulfuric acid 3% in ethanol.

Tannins has been highlighted by the solvent migration ethyl acetate-methanol-water (2: 1: 1) and revealed with aqueous solution of ferric chloride to 1%. Coumarins, emodols and anthracenosids were detected with migration solvent n-hexane-

ethyl acetate-toluene to 6: 2: 2 and revealed with the KOH solution (1 N).

Strains of *P. falciparum*

The biological material used was strains of *P. falciparum*, the species responsible for the majority of malaria cases in Africa. Strains of *P. falciparum* resistant to chloroquine K1 and sensitive to chloroquine 3D7 were used. The K1 parasites were provided by the laboratory Warhust, London School of Hygiene and Tropical Medicine, London, England, United Kingdom (LSHTM). The 3D7 parasites were provided by the Laboratory Nuguchi Memorial Institute on Medical Research (NMIMR) (Ghana). They were maintained in continuous culture in human blood in the Laboratory of "Centre National de Recherche et de Formation sur le Paludisme" (CNRFP) in Burkina Faso (Ouagadougou).

Continuous culture of parasites *in vitro* by the method of Trager

The strains in continuous culture were maintained using the technique of Trager and Jensen (1976) and we renewed the culture medium every 24 h. Parasites were thawed and cultured in flasks containing complete culture medium composed of RPMI 1640, L-glutamine 2%, Stamps Hepes 2%, Gentamicin 0.5%, Albumax 5% and Hypoxanthine 0.5%. The flasks containing the culture were aerated with mixed gas composed of 2% O_2, 5% CO_2, and 93% N_2. The flasks were then incubated in the CO_2 incubator at 37°C under conditions for maximal growth. Parasitaemia was controlled by making blood smears after the renewal of the culture medium. When parasitaemia reached 6%, a subculture was made using fresh blood without the interference from blood group A+.

***In vitro* evaluation of antiplasmodial activity**

The antiplasmodial activity of extract from *V. cinerea* Less was evaluated using the technique of *Plasmodium* Lactate Deshydrogenase (pLDH). Reference products (Dihydroartemisinin and Chloroquine) and extracts were dissolved in dimethylsulfoxide (DMSO) or in distilled water. The starting concentration of the extracts was 10 mg/ml that was further diluted to reach a final concentration of 100 µg/ml. The tests were performed on 96-well plates filled with a fixed volume of parasitized erythrocytes (2% parasitaemia). Samples were serially diluted with complete culture media (RPMI 1640 with albumax) to achieve the required concentration with DMSO concentration < 0.5%. Each extract was applied in a series of duplicate dilutions (final concentrations ranging from 0.78 to 50 µg/ml) on two rows. Dihydroartemisinin was used to validate the malaria test and chloroquine diphosphate salt (Sigma Aldrich) was used to validate the real chloroquine resistance of malaria strain K1. Infected and uninfected erythrocytes A+ were used as positive and negative controls, respectively.

After 72 h of incubation, the plates' counterpart's tests plates were prepared and the various substrates and coenzyme were then added. 100 µl MALSTAT (160 ml distilled H_2O, 200 µl Triton X100, 2 g of L-Lactate, 0.66 g Trizma base, 66 mg 3-acétylpiridine adenine di-nucleotide (APAD), at pH 9), 25 µl NBT/PES (100 ml of distilled water, 160 mg of NTB and PES 8 mg) and 20 µl of blood from the test plate was dispensed in each well including positive and negative controls. After 10 min of incubation, the plates were read on a spectrophotometer at a wavelength of 650 nm. Data were scored and analyzed using Microsoft Excel 2007. The mean optical density of negative controls was subtracted from that of each product to obtain the percentage of viability.

$$\% \text{ viability} = \frac{\text{OD product} - \text{OD negative control}}{\text{OD positive control}} \text{ X } 100 \quad (2)$$

The 50% inhibitory concentrations (IC_{50}) were calculated graphically with the Table Curve 2D v.5.0 software using the percentages of viability or cells proliferation versus log concentration. The IC_{50} of various extracts obtained were analyzed according to the criteria Deharo (Deharo et al., 2001); good activity IC_{50}<= 5 µg/ml, moderate activity 5<IC_{50}≤10 µg/ml, and inactive IC_{50}>10 µg/ml.

RESULTS AND DISCUSSION

Five types of extracts were prepared. Phytochemical screening of *V. cinerea* Less revealed the presence of alkaloids, triterpenes and sterols, saponins, tannins, emodols, anthracenosids, coumarins, and carotenoids (Tables 1 and 2). The *in vitro* antiplasmodial activity of the crude extracts on strains reference K1 and 3D7 was assessed by using five crude extracts of plant prepared. Amongst the 5 extracts tested, alkaloids extracts were identified as having good antimalarial effects (IC_{50}< 5 µg/ml), CH_2Cl_2 with moderate effects (5 µg/ml ≤ IC_{50}< 10 µg/ml), and CH_3OH, CH_3OH/H_2O and H_2O as inactive (IC_{50} ≥ 10 µg/ml) (Table 3) according to Deharo et al. (2001). The best antimalarial effects were obtained with alkaloids extracts of plant (Figures 1 and 3).

The crude alkaloids extracts from the whole plant showed good antimalarial effects against the chloroquine-resistant strain K1, with IC_{50} values 2.56 µg/ml. The moderate antimalarial effects were obtained with dichloromethane extracts against K1, with IC_{50} values 5.85 µg/ml (Figures 2 and 4).

In Cambodia, a similar study showed that dichloromethane extracts of *V. cinerea* had an IC_{50}= 18.3 µg/ml with the W2 malaria strain chloroquine-resistant (Hout et al., 2006). Although, our results are different from those of Simonsen et al. (2001) in India on ethanol extracts (82 µg/ml) tested on 3D7.

Based on Deharo's efficiency criteria, results from Cambodia and India are the same as our findings. These differences may be related to many parameters, including the local environment and the collection periods, which contribute to the variation of the chemical components of the plant as shown in a previous study on seasonal effects on bioactive compounds (Aires et al., 2011).

In Burkina Faso, a previous study showed that alkaloids extracts of bark of *Terminalia avicennoides* had an IC_{50}= 2.9 µg/ml with the K1 malaria strain (Sanon et al., 2013). Based on Deharo's efficiency criteria, results from this plant are a same from our findings.

The previous study was conducted with *Dicotoma tomentosa* (Asteracea) also collected in Burkina Faso, and in a different area. The antiplasmodial activity obtained with this plant (IC_{50}= 1.9 ± 0.2 µg/ml) was different from our dichloromethane extracts based on Deharo's efficiency criteria (Jansen et al., 2012).

In summary, our study confirms the pharmacological

Table 1. Summary of phytochemical screening of plant extracts was made according to Ciulei method (1982).

Chemical groups	Hydrolyzed extracts			Crude non-hydrolyzed extracts			
	CH_3OH	CH_3OH/H_2O	H_2O	CH_3OH	CH_3OH/H_2O	H_2O	CH_2Cl_2
Alkaloids	nr	nr	nr	–	–	–	–
Saponosids	nr	nr	nr	+	+	+	nr
Flavonoids	–	–	–	nr	nr	nr	–
Triterpenes/Sterols	+	+	+	nr	nr	nr	+
Tannins	nr	nr	nr	+	+	+	nr
Anthracenosids	+	–	–	nr	nr	nr	nr
Anthocyanosids	–	–	–	nr	nr	nr	nr
Emodols	nr	nr	nr	nr	nr	nr	+
Carotenoids	nr	nr	nr	nr	nr	nr	+
Coumarins	+	+	–	nr	nr	nr	+
Reductors compounds	nr	nr	nr	–	–	–	nr

(+): Presence ; (–) : not detected; (nr): not researched

Table 2. Chemical groups showed by chromatographic analysis thin layer.

Chemical groups	Hydrolyzed extracts			Crude extracts non-hydrolyzed				
	CH_3OH	CH_3OH/H_2O	H_2O	CH_3OH	CH_3OH/H_2O	H_2O	CH_2Cl_2	Alkaloids extracts
Alkaloids	nr	nr	nr	nr	nr	nr	nr	+
Tannins	nr	nr	nr	+	+	+	nr	nr
Triterpenes/Sterols	+	+	+	nr	nr	nr	+	nr
Coumarines	–	–	nr	nr	nr	nr	+	nr
Emodols	nr	nr	nr	nr	nr	nr	+	nr
Anthracenosids	+	nr	nr	nr	nr	nr	nr	nr
Saponosids	nr	nr	nr	–	–	–	nr	nr

(+): Presence ; (–) : not detected; (nr): not researched

Table 3. *In vitro* antiplasmodial activity of crude extracts obtained from *Vernonia cinerea* Less.

Plant	Family	Herbarium voucher	Part of plant	Extracts	Yield (in the plant) (%)	IC_{50} 3D7 (µg/ml)	IC_{50}K1 (µg/ml)
***Vernonia Cinerea* Less**	Asteracea	Cnrfp10Vc	Whole plant	CH_2Cl_2	2.57	8. 42	5.85
				CH_3OH	5.07	26.43	21.08
				CH_3OH/H_2O (1/1)	7.11	>50	41.56
				H_2O	23.7	>50	37.17
				Alkaloids extracts	0.2	4. 25	2.56
Chloroquine	-	-	-	-	-	0. 045	0.126
Dihydro-artemisinin	-	-	-	-	-	0. 0015	0. 002

properties of this plant species shown by its antibacterial activity with petroleum ether and ethanol extracts (Somasundaram et al., 2010), antipyretic, analgesic and anti-inflammatory activity (Iwalewa et al., 2003). Another study showed a good antiplasmodial activity of vernolide C and D molecules against W2 (Chea et al., 2006).

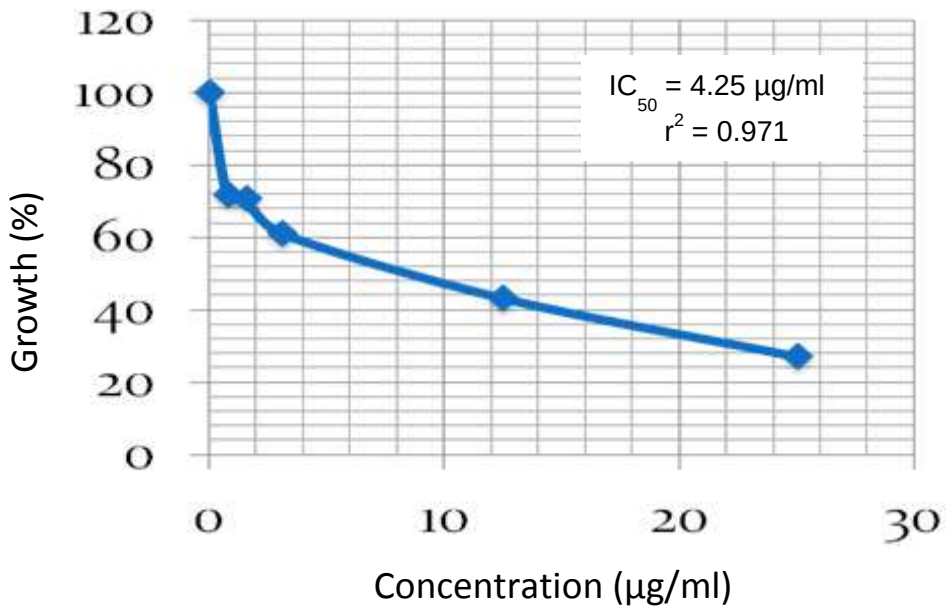

Figure 1. Curve of growth inhibition 3D7 of crude alkaloids.

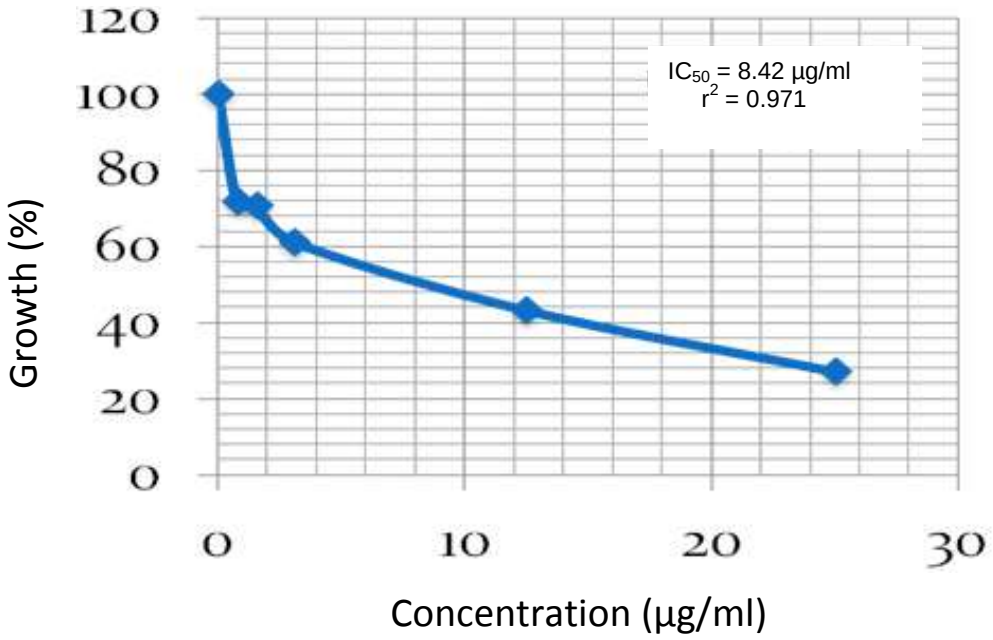

Figure 2. Curve of growth inhibition 3D7 of CH_2Cl_2 extracts.

This activity could be due to the presence of alkaloids (Bruneton, 1993) and triterpenes in the plant which mentioned by Chea et al. (2006). Alkaloids are one of the most important classes of natural products providing drugs since ancient times. The outstanding example is quinine from *Cinchona succirubra* (Rubiaceae) used for the treatment of malaria for more than three centuries (Kaur et al., 2009). Several plants of the Asteraceae family have been revealed as a good source for antimalarials. The most famous one is *A. annua*, the Chinese herb from which artemisinin (qinghaosu) was isolated (Liu et al., 1992). The good activity observed from the present investigation with *V. cinerea* Less which is a member of this family, thus supports the use of this

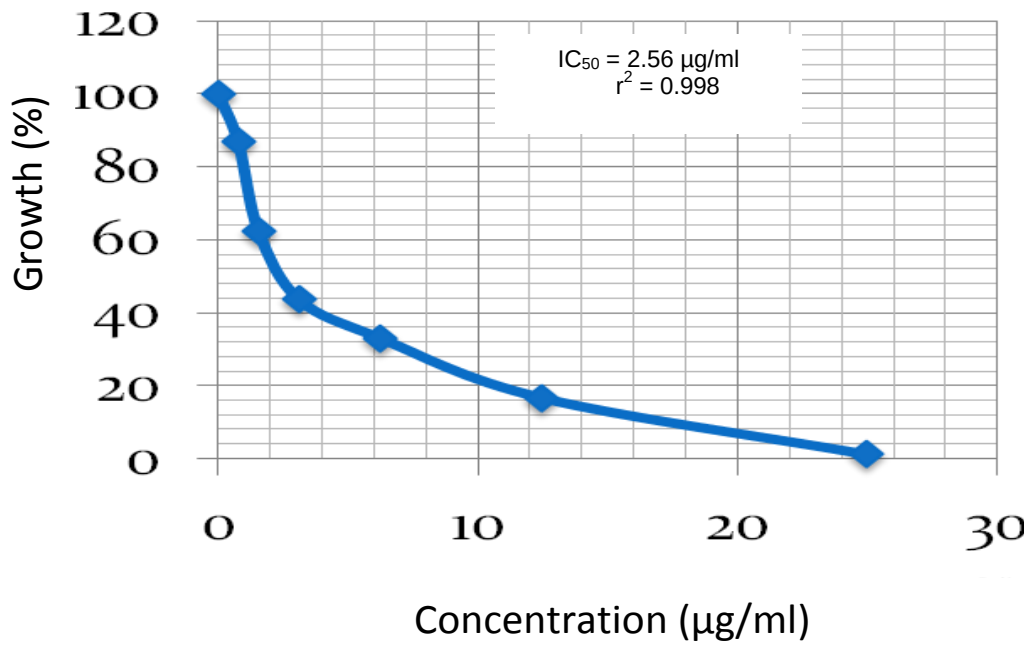

Figure 3. Curve of growth inhibition K1 of crude alkaloids extracts.

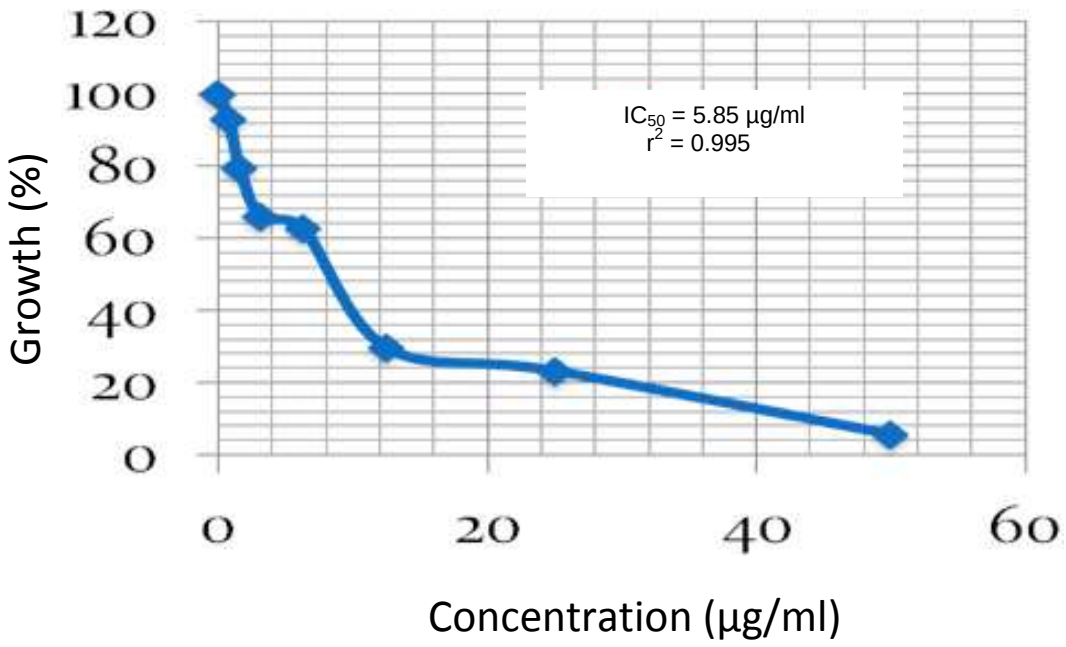

Figure 4. Curve of growth inhibition K1 of CH_2Cl_2 extracts.

plant for malaria in traditional medicine. This plant can be used in traditional medicine by paying close attention to the dosage. Further investigations are needed to evaluate the antiplasmodial activity *in vivo* in mice infected with *Plasmodium berghei* and to study the acute toxicity of plant extracts of *V. cinerea* Less, for the development of new drugs.

CONCLUSION

In vitro tests conducted on the parasites in the present

study have shown that *V. cinerea* Less has a very promising antiplasmodial activity. The CH_2Cl_2 extracts and crude alkaloids allowed us to get good IC_{50} on plasmodial strains. These results support the traditional use of this plant in traditional medicine for the treatment of malaria. Further studies will be needed, in particular *in vivo* tests on mice infected with *P. berghei* to assess antiplasmodial activity.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Aires A, Fernandes C, Carvalho R, Bennett RN, Saavedra MJ, Rosa ES (2011). Seasonal effects on bioactive compounds and antioxidant capacity of six economically important brassica vegetables. Molecules 16(8):6816-6832.

Batista R, De Jesus Silva Júnior A, De Oliveira AB (2009). Plant-derived antimalarial agents: New leads and efficient phytomedicines. part II. non-alkaloidal natural products. Molecules 14(8):3037-3072.

Bero J, Joëlle QL (2011). Natural products published in 2009 from plants traditionally used to treat malaria. Planta Med. 77:631-640.

Bero J, Michel F, Joëlle QL (2009). Antimalarial compounds isolated from plants used in traditional medicine. J. Pharm. Pharmacol. 61(11):1401-1433.

Bruneton J (1993). Pharmacognosie: phytochimie plantes médicinales (No. 581.634 B7).

Chea A, Hout S, Long C, Marcourt L, Faure R, Azas N, Elias R (2006). Antimalarial activity of sesquiterpene lactones from *Vernonia cinerea*. Chem. Pharm. Bull. 54(10):1437-1439.

Ciulei I (1982). Methodology for Analysis of Vegetable Drugs.Bucharest:Ministry of Chemical industry. p. 67.

Deharo E, Bourdy G, Quenevo C, Munoz V, Ruiz GS (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J. Ethnopharmacol. 77(1):91-99.

Dondorp AM, Nosten F, Yi P, Das D, Hanpithakpong W, Lee SJ, Lim P (2012). Artemisinin Resistance in *Plasmodium falciparum* Malaria. N. Engl. J. Med.361(5):455-467.

Hout S, Chea A, Bun SS, Elias R, Gasquet M, Timon-David P, Azas N (2006). Screening of selected indigenous plants of Cambodia for antiplasmodial activity. J. Ethnopharmacol. 107(1):12-18.

Iwalewa E, Iwalewa O, Adebayo J (2003). Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Less leaf. J. Ethnopharmacol. 86(2-3):229-234.

Jain SP, Puri HS (1984). Ethnomedicinal plants of Jaunsar-Bawar Hills, Uttar Pradesh, India. J. Ethnopharmacol. 12(213-222).

Jansen O, Tits M, Angenot L, Nicolas JP, De Mol P, Nikiema JB, Frédérich M (2012). Anti-plasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermal A-15- O-acetate as the main active compound. Malar. J. 11(1), 289.

Kaur K, Jain M, Kaur T, Jain R (2009). Antimalarials from nature. Bioorg. Med. Chem. 17(9):3229-3256.

Liu KCSC, Yang SL, Roberts MF, Elford BC, Phillipson JD (1992). Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. Plant Cell Rep. 11(12):637-640.

Nogueira CR, Lopes LMX (2011). Antiplasmodial natural products. Molecules 16(3):2146-2190.

Phillipson JD, Wright CW (1991). Can ethnopharmacology contribute to the development of antimalarial agents? J. Ethnopharmacol. 3*2*(1-3):155-165.

Rivière C, Nicolas JP, Caradec ML, Désiré O, Schmitt A (2005). Les plantes médicinales de la région nord de madagascar : une approche ethnopharmacologique. Ethnopharmacologia (36):36-48.

Sanon S, Gansane A, Ouattara LP, Traore A, Ouedraogo IN, Tiono A,Sirima SB (2013). *In vitro* antiplasmodial and cytotoxic properties of some medicinal plants from western Burkina Faso. Afr. J. Lab. Med. 2(1):1-7.

Sanon S, Ollivier E, Azas N, Mahiou V, Gasquet M, Ouattara CT, Fumoux F (2003). Ethnobotanical survey and in vitro antiplasmodial activity of plants used in traditional medicine in Burkina Faso. J. Ethnopharmacol. 86(2-3):143-147.

Simonsen HT, Nordskjold JB, Smitt UW, Nyman U, Palpu P, Joshi P, Varughese G (2001). *In vitro* screening of Indian medicinal plants for antiplasmodial activity. J. Ethnopharmacol. 74(2):195-204.

Somasundaram A, Velmurugan V, Senthilkumar GP (2010). *In vitro* Antimicrobial Activity of *Vernonia Cinerea* (L) Less. Pharmacologyonline-Newsletter 2: 957-960.

Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science 193(4254):673-675.

Traoré A, Derme AI, Sanon S, Gansane A, OuattaraY, Nebié I, Sirima SB (2009). Connaissances ethnobotaniques et pratiques phytothérapeutiques des tradipraticiens de santé de la Comoé pour le traitement du paludisme: processus d'une recherche scientifique de nouveaux antipaludiques au Burkina Faso. Ethnopharmacologia 43:35-46.

World Health Organization (WHO) (2008). World Malaria Report. Press, Geneva.

World Health Organization (WHO) (2010). Guidelines for registration of traditional medicines in the WHO African region.

World Health Organization (WHO) (2014). World Malaria Report. Geneva.

World Health Organization (WHO) (2015). World Malaria Report 2015.