Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(6): 143-153
Received: 23-09-2023
Accepted: 02-11-2023

Author's details are given below the reference section

Phytochemical characterization and evaluation of antiplasmodial activity of extracts of *Acanthospermum hispidum* harvested in Burkina Faso

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DOI: https://doi.org/10.22271/phyto.2023.v12.i6b.14777

Abstract
Acanthospermum hispidum is a plant belonging the Asteraceae family. In traditional medicine, it is used to treat pathologies with symptoms similar to that of malaria. The present work is a phytochemical characterization and antiplasmodial activities evaluation of *Acanthospermum hispidum* leaves extracts obtained with hexane, dichloromethane, ethyl acetate and methanol.
Antiplasmodial activity of the various extracts is evaluated on the *Plasmodium falciparum* strains 3D7, D10 and Dd2 according to the method described by Olivia Jansen *et al.* DCM extract of this plant showed good antiplasmodial activity on the D10 strain with an IC_{50} of 2.75 µg/mL and moderate antiplasmodial activity on the Dd2 strain with an IC_{50} of 7.82 µg/mL
Phytochemical screening showed the presence of flavonoids, alkaloids, saponins and tannins in most of extracts. The best contents of total phenolics, total flavonoids, total alkaloids and antioxidants are 155.79 mg GAE/g extract, 36.27 mg QE/g extract, 124.68 µg qE/g extract and 271.32 mg TE/g of extract respectively for the methanolic, dichloromethane, ethyl acetate and methanolic extracts.
The major flavonoids of the DCM extract, characterized by HPLC-MS, were identified as 3,4', 5, 7-tetrahydroxy-3',5'-dimethoxyflavone, myricetintrimethylether, 5-hydroxy-7,3',4',5'-tetramethoxyflavone 3-O-rhamnoside, Quercetin 3-O-rhamnoside and Kaempferol 3-O-glucoside.
Dichloromethane extract of *Acanthospermum hispidum* is a potential source of bioactive molecules and could use as a substrate in the formulation of new antimalarial drugs.

Keywords: *Acanthospermum hispidum*, phytochemistry, HPLC-MS, flavonoids, antiplasmodial activity

1. Introduction
The use of plants by men in several areas of daily life remains remarkable despite technological and modern progress. Plants have an important role in the human being life, especially in nutrition and medicine.
Plants are a potential source of phytomedicines because despite the considerable progress observed in the field of synthetic organic chemistry, more than 25% of the drugs prescribed in our health facilities derive directly or indirectly from plants [1]. They contain many metabolites such as phenolics and alkaloids which participate and/or reinforce the immune defense against oxidative stress, thus preventing the body against the various chronic pathologies and the cardiovascular diseases [2, 3].
Malaria, which is endemic in many countries in sub-Saharan Africa, could be effectively suppressed despite the various resistances developed by *Plasmodium falciparum* [4].
Many cases parasitic resistance to quinine [5], amodiaquine [6] and mefloquine [7] have been reported despite the success of chloroquine to fight against malaria-related mortality [8, 9].
Today, *Plasmodium falciparum* remains resistant to many antimalarial drugs used, even to the most recent such as associations based on artemisinin extracted from Artemisia annua and considered in 2001 by the World Health Organization as "the greatest hope against malaria" [10, 11].
The search of plant extracts with antiplasmodial activity is still necessary to fight against incessant resistance and to cope with the high cost of drugs for most households in sub-Saharan Africa [4].
Acanthospermum hispidum was targeted in this present study because it is widely useed in the field of traditional medicine [12, 13]. According to Sanon S. *et al.*, Appiah-opong R *et al.*, aqueous extract, chloroform, methanolic and lactone extracts of the leaves of this plant showed significant antiplasmodial activities on 3D7 and W2 strains [14, 13].

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However, little studies, were carried out on phytochemical and antimalarial activities on Dd2 and D10 of extracts from different solvents of leaves of *Acanthospermum hispidum* harvested in Burkina Faso.

The present work aims to realize a phytochemical characterization then to evaluate the antiplasmodial activity of the extracts of the leaves of *Acanthospermum hispidum* harvested in Burkina Faso for a possible valorization as phytomedicine.

2. Materials and Methods

2.1. Materials

2.1.1 Plant material

The plant material included *Acanthospermum hispidum* leaves, harvested in August 2020 in the Gondologo area in Ouahigouya, in Burkina Faso. After harvest, the leaves were washed, dried and then powdered.

2.1.2 Biological material

Strains of *Plasmodium falciparum* were strains 3D7 (Chloroquino sensitive), D10 (Chloroquino sensitive) and Dd2 (Chloroquino resistant). These strains were grown in complete culture media (Albumax II 1% in Roswell Park Memorial Institute (RPMI) medium) and mixed gas (2% O2, 5% CO2 and 93% N2) at the laboratory of pharmacognosy at the National Malaria Research and Training Center (NMRTC) in Ouagadougou, Burkina Faso. A smear is made and then stained with Giemsa to determine the parasite density of each culture and check the state of the culture for possible contamination.

2.2. Methods

2.2.1. Extracts preparation

100 g of the powder of *A. hispidum* were extracted by maceration with 300 mL of solvent using successively the hexane, the dichloromethane, the ethyl acetate and the methanol. This operation is repeated three times. Before switching to the next solvent, the residue was dried for 24 hours. The extracts were evaporated to dryness with rotary evaporator then keep in the freezer.

2.2.2. Phytochemical screening

Phytochemical screening was carried out using thin layer chromatography (60 F254, support-aluminum, 20×20, Fluka-Silica/silica gel) and referring to the literature [2, 15]. Depending on the type of secondary metabolites to be identified, specific reagents as well as different appropriate solvent systems have been used (Table 1) [16, 17].

Table 1: Chemical groups highlighted by thin layer chromatography (TLC) [16, 17].

Chemical groups	Solvent systems	Detection reagents	Observed fluorescence
Flavonoids	Ethyl acetate/formic acid/glacial acetic acid/water (100:11:11:26 v/v)	Neu reagent (2-amino-ethyl-diphenylboronate + Polyethylene glycol (PEG) 4000)	Blue, Yellow, Orange
Tannins	Water / methanol / butanol / glacial acetic acid / (3.5:1.25:10:1.25 v/v)	FeCl3 at 2% (m/V) in a hydroalcoholic solution (50%)	Greenish-blue, blackish-green
Alkaloids	Toluene/ ethyl acetate / diethylamine (70:20:10 v/v)	Dragendorff's reagent (Potassium tetraiodobismuthate)	Orange
Saponins	Hexane/ ethyl acetate /methanol/ (35:10:5)	sulfuric anisaldehyde then heat to 110°C	Red-violet
Carotenoids	Petroleum ether / acetone/ dichloromethane (3:1:1)	None	Yellow-orange

2.2.3. Evaluation of total phenolics content (TPC)

The total phenolic content was evaluated by the method using the Folin-Ciocalteu reagent as described by Singleton *et al.* [16].

A volume of 60 µL of the Folin-Ciocalteu reagent (diluted 10 times) was added to 60 µL of each suitably diluted extract and allow to stand for 8 minutes et 25 °C. Then, 120 µL of 7.5% sodium carbonate solution were added to the reaction mixture. 30 minutes after incubation for 30 min at a temperature of 37 °C, the absorbances were measured at 760 nm.

Gallic acid was used to establish the calibration curve. The contents of total phenolics were determined by relating the absorbances to the equation obtained from the calibration curve ($y = 23.538x + 0.1028$; $R^2 = 0.995$) and the results were expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g of extract) [18].

2.2.4. Evaluation of total flavonoids contents (TFC)

Total flavonoids contents was estimated according to the method described by Woisky and Salatino [18].

In this method, 120 µL of a 2% $AlCl_3$ solution in ethanol were added to 120 µL of each suitably diluted extract. The absorbance was measured at 420 nm, after 1 hour of incubation at room temperature. Total flavonoids contents were calculated from equation of calibration curve of the quercetin ($y = 44.639x + 0.0263$; $R^2 = 0.998$). Results were expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

2.2.5. Evaluation of total alkaloids contents

Total alkaloid content was determined according to the method described by Manjunath Ajanal *et al.*, [19]. 1 mL of the acidified methanol extract (2N HCl) with a concentration of 40 mg/mL was taken and filtered. The pH is adjusted to neutral with 0.1 N NaOH solution. To the neutral filtrate, 5 mL of bromocresol green with a concentration of 69.8 mg/L and 5 mL of phosphate buffer (2 M) are added in a separating funnel. The mixture was stirred vigorously, and the complex formed is extracted by successive exhaustion with 1, 2, 3 and 4 mL of chloroform. A total volume of 10 mL of chloroform extract is obtained. The absorbance of the extracts is read at 470 nm. Quinine was used to draw a calibration curve ($y = 0.0291x + 0.056$; $R^2 = 0.997$). The total alkaloid contents are expressed in micrograms of quinine equivalent per g of extract (µg qE/g).

2.2.6. Evaluation of total antioxidants contents

Antioxidants contents of each extract is determined using the radical DPPH• and according to the method as described by Brand-Williams *et al.*, [20].

120 µL of the methanolic solution of DPPH• at 4. 10^{-2} mg/mL were added to each suitable diluted solution of the various extracts. After 10 minutes of incubation 25 °C in darkness. The absorbances were read at 510 nm with a SAFAS type spectrophotometer. The antioxidant contents were obtained by relating the absorbances of the extracts to the calibration curve ($y = - 44.084x + 0.5434$; $R^2 = 0.997$) established using trolox. The results were expressed in mg of trolox equivalent per gram of extract (mg TE/g).

2.2.7. Evaluation of antiplasmodial activity

Antiplasmodial activity was made using the method described by O. Jansen *et al.*, and M. L. Willcox *et al.*, [21 22]. From the extract stock solutions, suitable diluted solutions (50 µg/mL to 0.78 µg/mL) were prepared using 100 µL of complete culture medium (CCM) in a flat-bottomed 96-well microplate (test plate). Then 100 µL of 2% parasitized blood were added to the extracts at different concentrations to afford a 200 µL final volume of 2% parasitaemia blood. Each extract was tested in triplicate.

Control samples were also prepared:

- A parasite growth control or positive control containing no extract.
- A negative control composed of healthy red blood cells to determine the auto-fluorescence threshold.

The microplates containing the mixture of parasitized blood, the various extracts and the controls are placed in a chamber (The jar). The jar was fed with mixed gas (favorable conditions to parasitic growth) and placed in a CO_2 incubator at 37 °C for 72 hours.

After incubation, a new 96-well plate arranged in the image of the test plate is made. 100 µL of the MALSAT solution (Triton X100+L-Lactato+Trizma base) were introduced into all the wells. Then, 20 µL of the NTB/PES solution (Nitro tetrazolium Blue Chloride / Phenazine ethosulfate) were added in the dark. 25 µl of the content (Parasited blood and the extracts) of each homogenized well of the test plate incubated for 72 hours were again added to each well of the new plate. At the end of a new incubation for 10 minutes at 25 °C and away from the light of the new mixture, the absorbances of the resulted mixture were read at 650 nm using an ELx808-BIOTEX type spectrophotometer [21, 22].

The results were expressed in Inhibitory Concentration of 50% (IC_{50}) values of parasites proliferation using the software Table Curve 2D jandel Scientific version 3.0.

The classification scale of the results of the evaluation of antiplasmodial activity is shown in Table 2.

Table 2: grid for *in vitro* antiplasmodial activity evaluation according to Willcox M and al., [22].

IC_{50} (µg/mL)	Antiplasmodial activity
< 2.0	**Very good**
2.0-5.0	**Good** This is the concentration range generally considered to be active in screening programs for antimalarial activity, justifying bioassay-guided fractionation.
5.1-10	**Moderate** This range can reasonably be considered for bioassay-guided fractionation.
11-25	Low
26-50	Very low
> 50	Inactive

2.2.8 Evaluation of hemolytic activity

The *in vitro* hemolysis test is performed according to the method described by O. Jansen and al., [21].

A volume of 5 ml of blood previously preserved in an ice bath is washed with the PBS (Phosphate Buffer Saline), at pH 7.4 and then centrifuged at 2200 rpm at 4 °C for 15 minutes. The supernatant was removed and the washing process was repeated 2 times on the pellet. After washing, the optical density is adjusted to 1.5 at the wavelength of 550 nm and the red blood cells are diluted at 10% (V/V) in PBS at room temperature.

As for the analysis of the viability of the red blood cells before the evaluation of hemolytic activity of the extracts, a cryotube was filled with 10 µL of PBS (Phosphate Buffer Saline) and another one with 10 µL of triton X-100 at 20% (V/V) in distilled water. In each tube was then completed to 200 µL with red blood cells.

After 3 minutes of centrifugation at 2200 rpm, the tube containing the triton mixture shows no pellet; on the other hand, the tube containing the mixture of PBS has a pellet at the bottom of the cryotube.

The first cryotube containing the Phosphate Buffer Saline solution is the negative control and the second cryotube containing the triton X-100 is the positive control.

For the hemolytic test of the extracts, a stock solution of each sample to be tested was prepared in the RPMI at 100 µg/mL and 50 µg/mL. 10 µL of the stock solution at the two different concentrations are each placed in a tube and then 190 µL of 10% red blood cells are added. The mixtuaare is incubated for 1 hour at room temperature. Then the tubes are centrifuged for 5 minutes at 2200 rpm. At the end of the centrifugation, 150 µL of the supernatant are placed in a well of the 96-well reading plate and the absorbances are read at 550 nm with the spectrophotometer [21]. The percentage of hemolysis is calculated according to the formula:

% Hemolysis = (A (sample tested) - A (negative control))/ (A (positive control) - A (negative control))

A is the absorbance at 550 nm.

3. Major flavonoids characterization by HPLC/MS

The major flavonoids of DCM extract were analyzed by High Performance liquid chromatograph coupled to the Mass Spectrometer (HPLC-MS). The experimental device was consisted of High-performance liquid chromatograph type HP-1200 equipped with a quaternary pump, an auto sampler, a Diode Array Detector (DAD) and an API-electrospray quadrupole type MS-6110. The positive ionization mode has been applied. The column used is of Kinetex XB-C18 type from Phenomenex, (Length: 150 mm, internal diameter: 4.5 mm; particle size: 5 µm).

The solution A, using as mobile phase, was water acidified with 0.1% formic acid and the solution B was acetonitrile acidified with 0.1% formic acid. The multi-step linear gradient was applied: 5% solution B for 2 minutes; then the proportion of solution B is varied from 5% to 90% over the following 18 minutes and then the proportion of 90% of solution B is maintained for 4 minutes. Finally, for 6 minutes the proportion of solution B is reduced to 5%. The flow was 0.5 mL/min at 25 ± 0.5 ^{0}C.

The analysis time lasted dure 30 min and the chromatograms were recorded at wavelengths λ = 280 nm and λ = 350 nm.

Scan mode in mass spectrometry was used to detect positive ions.

The experimental conditions applied were

- The temperature of nitrogen gas was equal to 350.0 °C with a flow rate of 7 L/min,
- The nebulizer pressure was 35 Psi (Pound per Square inch) or 2.413 bars, and the capillary voltage was equal to 3000 V,
- The fragmenters had a voltage of 100 V and the mass to charge ratios of the ions (m/z) were recorded between 120 -1500.

The structural identification of the compounds is carried out by referring to the following database Polyphenols-Phenol-Explorer and using the data from literature [23].
The molecular masses of the parent ions were searched for in this bass after an indication through the spectrum UV and that this compound could belong to the flavonoid family.

4. Results and Discussion

4.1. Extracts preparation

The extraction yields are reported in Table 3. A total of four extracts were obtained and the extraction yields vary between 0.68 and 9.43%.
The methanolic extract gave the best extraction yield with a value of 9.43%. The lowest extraction yield (0.68%) is obtained for the ethyl acetate extract with a yield of the compounds present in the plant material used have a much greater affinity with methanol which is a polar solvent than ethyl acetate. The hexane and dichloromethane extracts have extraction yields of 1.18 and 2.03% respectively. These yields are low compared to the extraction yield with methanol but remain greater than that with ethyl acetate. The extraction yields listed in Table 3 provide information on the quantity of plant material to be made available for a substantial extraction.

4.2. Phytochemical screening

Results of phytochemical screening by thin layer chromatography (TLC) are recorded in Table 3. The groups of compounds highlighted in the extracts studied are carotenoids, flavonoids, tannins, saponins and alkaloids. The methanolic and ethyl acetate extracts contain all groups of compounds sought. Dichloromethane extract indicates the presence of compounds except flavonoids and tannins. As for the hexane extract, it only contains saponins and carotenoids. The unique presence of tannins and flavonoids in the methanolic and ethyl acetate extracts could be due to the polarity of these compounds. In Odisha (India), Harekrishna Roy *et al.* also reported that *Acanthospermum hispidum* extracts obtained using chloroform, hydroalcoholic (70%) and petroleum contain terpenoids, alkaloids, glycoside, tannins (chloroform) and saponins, tannins, flavonoids glycoside and alkaloids (Hydroalcoholic) [24].

Table 3: Extraction yields and chemical groups highlighted in different extracts of *Acanthospermum hispidum*.

Extracts of *Acanthospermum hispidum*	**Extraction yields** (%)	**Highlighted chemical groups**				
		Flavonoids	**Alkaloids**	**Tannins**	**Saponins**	**Carotenoids**
Hexane	1,98	-	-	-	+	+
Dichloromethane	2,03	-	+	-	+	+
Ethyle acetate	0,68	+	+	+	+	+
Methanol	9,43	+	+	+	+	+

-: absence, +: presence

4.3. Total phenolics content (TPC)

The total phenolic content of all the extracts varied between 22.38 and 155.79 mg GAE/g of extract (Table 4). The results obtained show that the methanolic extract is the richest in total phenolic compounds (155.79 mg GAE/g of extract), followed by the ethyl acetate extract (56.37 mg GAE/g of extract). The dichloromethane and hexane extracts contents are respectively 28.44 and 22.38 mg GAE/g of extract.
Remy K Bationo *et al.*, showed that the methanol extract of the leaves of *Cymbopogon giganteus* contained the best content of total phenolic compounds with a content of 132.95 mg GAE/g of extract [25]. The same study showed that DCM extracts from different parts of the plant contained the lowest levels of total phenolic compounds [25]. The contents of total phenolic compounds are in the same increasing order as the polarity of the solvents, which is in agreement with our results. According to the work of Kouassi and al., the methanolic extracts of the leaves and flowers of *C. giganteus* contained the best levels of total phenolic compounds (118.14 mg GAE/g of extract) [26]. The phenolic compound contents of the methanolic and ethyl acetate extracts of *Acanthospermum hispidum* remain higher than those reported by Yougoubo Abdoulaye *et al.* in this work on the leafy branches of *G. maderaspatana* where the contents were 57.921 and 12.260 mg GAE/g of extract respectively for the ethyl acetate and methanol extracts [27].

4.4. Total flavonoids contents (TFC)

Total flavonoid contents expressed in milligrams quercetin equivalent per gram of extract (mg QE/g) ranger between 5.07 and 36.27 mg QE/g for the extracts. The dichloromethane extract of *Acanthospermum hispidum* recorded the highest content (36.27 mg QE/g of extracts) followed by that of ethyl acetate (Table 4).
Similar studies conducted by Remy K Bationo *et al.*, showed that the dichloromethane fractions of *Cymbopogon giganteus* stems and roots contained the best total flavonoid contents of 207.20 and 348.34 mg QE/g extracts respectively. These values are higher than those of our work [25]. In addition, Yougoubo *et al.*, showed that the ethyl acetate fractions were the richest in flavonoids followed by the dichloromethane fractions on all the different parts of *G. maderaspatana* [27].

4.5. Total alkaloid content (TAC)

Total alkaloid contents are presented in Table 4.
The ethyl acetate extract of *Acanthospermum hispidum* contains the most alkaloids with contents (124.68 µg qE/g of extract). The dichloromethane extract of the leaves also contains alkaloids with a content up to 87.43 µg qE/g of extract. The total alkaloid contents of the other extracts are the lowest. They vary randomly depending on the extraction solvent and are 14.49 µg qE/g of extract for the methanolic extract and 44.99 µg qE/g of extract for the hexane extract.

The alkaloids contained in the leaves of *Acanthospermum hispidum* are much more extractable with ethyl acetate and dichloromethane than the other solvents used (methanol and hexane).

The total alkaloid contents of the various extracts from the leaves of *Acanthospermum hispidum* remain low compared to those of the methanolic extracts of the roots of *Plumbago zeylanica Linn* (269.5 µg Atropine E /g of extract) and *Piper longum Linn* (140.7 µg Atropine E / g of extract) reported by Manjunath Ajanal and al., [19]. On the other hand, the ethyl acetate extract of the leaves of *Acanthospermum hispidum* contains more alkaloids (124.68 µg qE/g of extract) than the methanolic extract of the stems of *Rhum emodi Wall* (106.5 µg Atropine Equivalent / g of extract) [19].

4.6. Antioxidant content (AOC)

Total antioxidant contents of the different extracts are grouped together in Table 4. The contents vary between 4.58 and 271.32 mg TE per g of extract. The methanolic and ethyl acetate extracts of *Acanthospermum hispidum* have the best total antioxidant contents of 271.32 and 137.98 mg TE/g of extract respectively. Dichloromethane and hexane extracts are low in antioxidant content; the lowest content is observed in the dichloromethane extract with a content of 4.58 mg TE/g of extract.

Extracts with more polar solvents contain more antioxidants than extracts with less polar solvents. Extracts rich in phenolic compounds and flavonoids generally have the best antioxidant content [28]. In the present work, the methanolic extract which contained the best content of total phenolic compounds also contained the best content of total antioxidants, followed by the ethyl acetate extract in the same order. These results are in agreement with the work of Remy K Bationo and al., [25].

In addition, the work of Yougoubo Abdoulaye and al., carried out on the extracts of the leafy branches of *G. maderaspatana* showed that the ethyl acetate extract contained the best content of total phenolic compounds (57.921 mg GAE/g of extract) followed by the methanol extract (12.260 mg GAE/g extract) and the antioxidant levels were in the same order with 13.209 and 11.355 mg TE/g extract respectively [27].

Table 4: total phenolics, flavonoids, alkaloids and antioxidant contents of *Acanthospermum hispidum* leaves extracts.

Extracts of *Acanthospermum hispidum*	TPC (mg GAE/ g extract)	TFC (mg QE/ g extract)	TAC (µg qE/ g extract)	AOC (mg TE/ g extract)
Hexane	22,38 ± 1,15	5,07 ± 0,31	44,99 ± 2,12	5,91 ± 0,98
Dichloromethane	28,44 ± 3,84	36,27 ± 1,97	87,43 ± 2,84	4,58 ± 0,28
Ethyle acetate	56,37 ± 2,50	21,41 ± 1,25	124,68 ± 4,12	137,98 ± 5,4
Methanol	155,79 ± 9,25	5,09 ± 0,31	14,49 ± 1,44	271,32 ± 7,92

4.7. Antiplasmodial activity

The Table 5 presents the resulteszs obtained at the end of the evaluation of the antiplasmodial activity.

Table 5: Antiplasmodial activities (IC_{50}) of *A. hispidum* on the sensitive strains 3D7 and D10 and on the resistant strain Dd2 and hemolysis rate.

Extracts of *Acanthospermum hispidum* and References	IC_{50} (µg/mL)			Hemolysis rate %
	Strains 3D7	Strains D10	Strains Dd2	
Hexane	18,16 ± 3,14	6,90 ± 1,02	15,59 ± 2,9	0,064
Dichloromethane	7,19 ± 0,69	2,75 ± 0,56	7,82 ± 0,88	0
Ethyl acetate	20,26 ± 2,57	10,81 ± 2,86	15,90 ± 4,09	0
Methanol	51,39 ± 0,08	52,42 ± 0,00	52,42 ± 0,00	0
Chloroquine	8,42 ± 0,50	6,39 ± 0,50	Nd	Nd
Dihydroartemisinin	Nd	Nd	0,71 ± 0,00	Nd

ND: No Determined

Analysis of the results in Table 5 according to a scale proposed by Wilcox *et al.* shows that the antiplasmodial activity of the different extracts on the two chloroquine-sensitive strains 3D7 and D10 of *Plasmodium falciparum* depends on the extraction solvents.

Indeed, the evaluation of the antiplasmodial activity carried out on the 3D7 and D10 strains of the four (04) extracts of *Acanthospermum hispidum* showed moderate antiplasmodial activity on the 3D7 strain for the dichloromethane extract with an IC_{50} of 7.19 µg /mL and good antiplasmodial activity on the D10 strain with an IC_{50} of 2.75 µg/mL. The hexane extract and the ethyl acetate extract gave low activities on the two strains with IC_{50} of 18.16 and 20.26 µg/mL respectively for the 3D7 strain and 6.90 and 35, 73 µg/mL for strain D10. The methanolic extract remained inactive on the two strains 3D7 and D10 with IC_{50} values greater than 50 µg/mL.

The dichloromethane extract is best indicated to fight against the two sensitive strains 3D7 and D10 of Plasmodium falciparum. It has an inhibitory activity comparable to that of chloroquine taken as a reference.

The inhibitory activity carried out on the resistant strain Dd2 of *Plasmodium falciparum* gave the results recorded in Table 5 above. The dichloromethane extract gave moderate antiplasmodial activity against this resistant strain with an inhibitory concentration of 7.82 µg/mL. The hexane extract as well as the ethyl acetate extract showed weak antiplasmodial activity on the Dd2 strain with IC_{50} values of around 15 µg/mL.

These results are in agreement with the work of Sanon S and al., which showed that the chloroform fraction of the aqueous extract of the leaves of *Acanthospermum hispidum* has a moderate antiplasmodial activity with an inhibitory concentration of 50% (IC_{50}) equal at 5.02 µg/mL on the W2 strain of *Plasmodium falciparum* [29].

These results are also similar to those of Bero J and al., who obtained an IC_{50} of 4.80 µg/mL on the same strain but with a dichloromethane extract [30]. Furthermore, the work of Ganfon H., in 2012, on the 3D7 strain using a lactonic extract, resulted in an IC_{50} of 2.33 µg/mL.

Many works have described in recent years that the antiplasmodial properties of various compounds of natural origin and more particularly of plant origin, derive from various types of alkaloids (bisbenzylisoquinolinic, furoquinolinic, acridose, indoloquinolinic) [31]. The two drugs (quinin; Artemisinin) currently most useful in antimalarial therapy, quinine is also part of alkaloids family [31]. We find

that the ethyl acetate and dichloromethane extracts which are rich in total alkaloids gave better antiplasmodial activities. There would then exist a relationship between the total alkaloid contents and the inhibitory concentrations of the different extracts on each of the three strains 3D7, D10 and Dd2.

The dichloromethane extract of *Acanthospermum hispidum*, whose total alkaloid content is 87.43 µg qE/g of extract, is the most active on all the strains tested, followed by the hexane extract (44.99 µg qE/g of extract) then ethyl acetate extract (124.68 µg qE/g of extract).

The dichloromethane extract remains the most active despite its relatively low total alkaloid content compared to the ethyl acetate extract. The alkaloids which have good antiplasmodial activity contained in the leaves of *Acanthospermum hispidum* are extracted with dichloromethane. This constant is in agreement with the work of Florence D. OUEDRAOGO who asserts that the dichloromethane extract of the leaves and flowers of G Senegalensis would contain harmane [32].

The methanolic extract remained inactive on the sensitive strains (3D7; D10) as well as on the Dd2 resistant strain of *Plasmodium falciparum*. The molecules active on the 3D7, D10 and Dd2 strains certainly do not have a good affinity with methanol.

4.8. Hemolytic activity

The evaluation of the hemolytic rate of the extracts gave the results recorded in Table 5.

All the extracts except the hexane extract showed a hemolysis rate of 0%. This shows that a possible use of these extracts would not negatively effect on the red blood cells. The dichloromethane and ethyl acetate extracts which showed good antiplasmodial activity, have no effect on the cells.

According to Laurencin *et al.*, and Olivier Jansen *et al.*, an extract has a hemolytic character when it hemolysis at least 5% of the red blood cells contained in a solution [21].

Referring to these works, all the extracts show no harmful hemolytic effect.

5. Phytochemical characterization by HPLC/MS

HPLC chromatograms were recorded successively at wavelengths of 280 nm and 350 nm corresponding to the absorption wavelengths of flavonoids [33, 34].

Chromatograms indicate 4 characteristic peaks identified as peaks 1, 2, 3, 4 respectively at the retention times t_R1 = 22.199 min, t_R2 = 23.667 min, t_R3 = 24.325 min and t_R4 = 24.679 min (Table 6) (Figure 2 et Figure 3). These peaks have the same retention time when recording the chromatogram at 280 nm as when recording the chromatogram at 350 nm [33] [34]. This proves that these compounds could be flavonoids.

Compound 1 (t_R1 = 22.199 min) exhibits a mass spectrum with an iron at m/z 247 which, following fragmentation, gave fragment ions at m/z of 183 and 153 (Table 6) (Figure 4). These ions are the result of fragmentation into $^{1.3}A$ and $^{0.2}B$ [35]. Moreover, the UV spectrum of compound 1 shows two signals at 253 nm and at 350 nm [33] [34]. These signals are characteristic of flavonoids which present two signals around the same wavelengths. Compound 1 is identified as 3, 4', 5, 7-tetrahydroxy-3', 5'-dimethoxyflavone ($C_{17}H_{14}O_8$) (Table 6) (Figure 1).

Compound 2 (t_R2 = 23.667 min) has an ion at m/z 361 (Figure 4). This ion following a loss of 3 mass units of 15 Da Each gives an ion at m/z 316 which is identified as myricetin. The UV spectrum of compound 2 shows two signals at 240 nm and at 350 nm which are characteristic of those of flavonoids [33, 34]. Compound 2 is identified as being myricetintrimethylether ($C_{18}H_{16}O_8$) (Table 6) (Figure 1).

Compound 3 (t_R3 = 24.3 min) has an ion at m/z 521 which by loss of a mass unit of 146 Da corresponding to a rhamnose ($C_6H_{12}O_4$, 148 Da) [36] gives another ion m/z 375 (Figure 6).

The product ion m/z 375 was formed from the structure of 5-hydroxy-7, 3', 4', 5'-tetramethoxyflavone ($C_{19}H_{18}O_8$, 374 Da). The UV spectrum shows two signals, one at 270 nm and the other at 340 nm [33, 34]. These signals are in the domain of the flavonoid absorption bands. Compound 3 is identified as 5-hydroxy-7, 3', 4', 5'-tetramethoxyflavone 3-O-rhamnoside ($C_{25}H_{28}O_{12}$) (Table 6) (Figure 1).

Compound 4 (t_R4 = 24.6 min) m/z 450 was defined as the parent ion and two product ions were formed. The neutral losses between m/z 450 and m/z 287 and between m/z 450 and m/z 303 were 163 and 147 Da respectively, which means that compound 4 could have a glucose structure or a rhamnose structure (Fig. 7) [36]. The product ion m/z 287 was formed from the structure of kaempferol (kaempferol, $C_{15}H_{10}O_6$, 286 Da) and the product ion m/z was formed from the structure of quercetin ($C_{15}H_{10}O_7$, 302 Da). The UV spectrum shows three signals at 255, 270 and 350 nm which are characteristic of those of flavonoids [33] [34].

Compound 4 could be identified as either quercetin 3-O-rhamnoside ($C_{21}H_{20}O_{11}$) (Table 6) (Figure 1) or kaempferol 3-O-glucoside ($C_{21}H_{20}O_{11}$) (Table 6) (Figure 1).

Table 6: HPLC/MS analysis results of dichloromethane extract of *Acanthospermum hispidum*

Retention time	Wavelength	m/z of parent ion	Loss of mass units	Raw formula	Molecule name
22,19	253; 350	247	153; 183	$C_{17}H_{14}O_8$	3,4',5,7-tetrahydroxy-3',5'-dimethoxyflavone
23,66	240; 350	361	3 x 15	$C_{18}H_{16}O_8$	myricetintrimethylether
24,3	270; 340	521	146	$C_{25}H_{28}O_{12}$	5-hydroxy-7,3',4',5'-tetramethoxyflavone 3-O-rhamnoside
24,6	255; 270; 350	450	147	$C_{21}H_{20}O_{11}$	quercetin 3-O-rhamnoside
24,6	255; 270; 350	450	163	$C_{21}H_{20}O_{11}$	kaempferol 3-O-glucoside

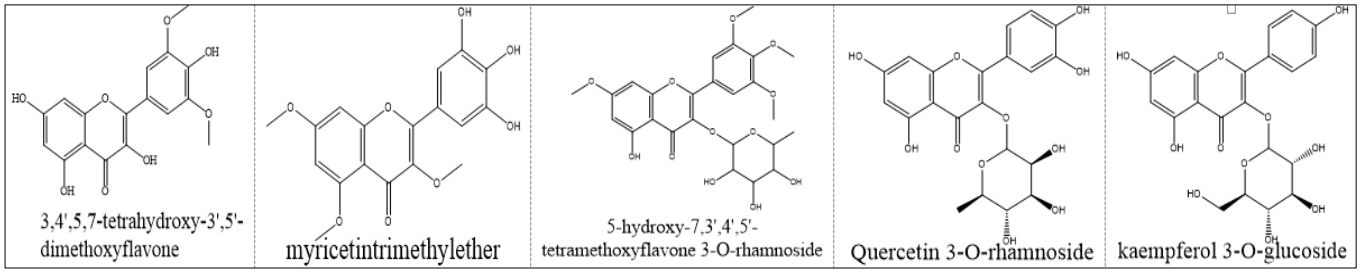

Fig 1: Names and formulas of five identified molecules in dichloromethane extract

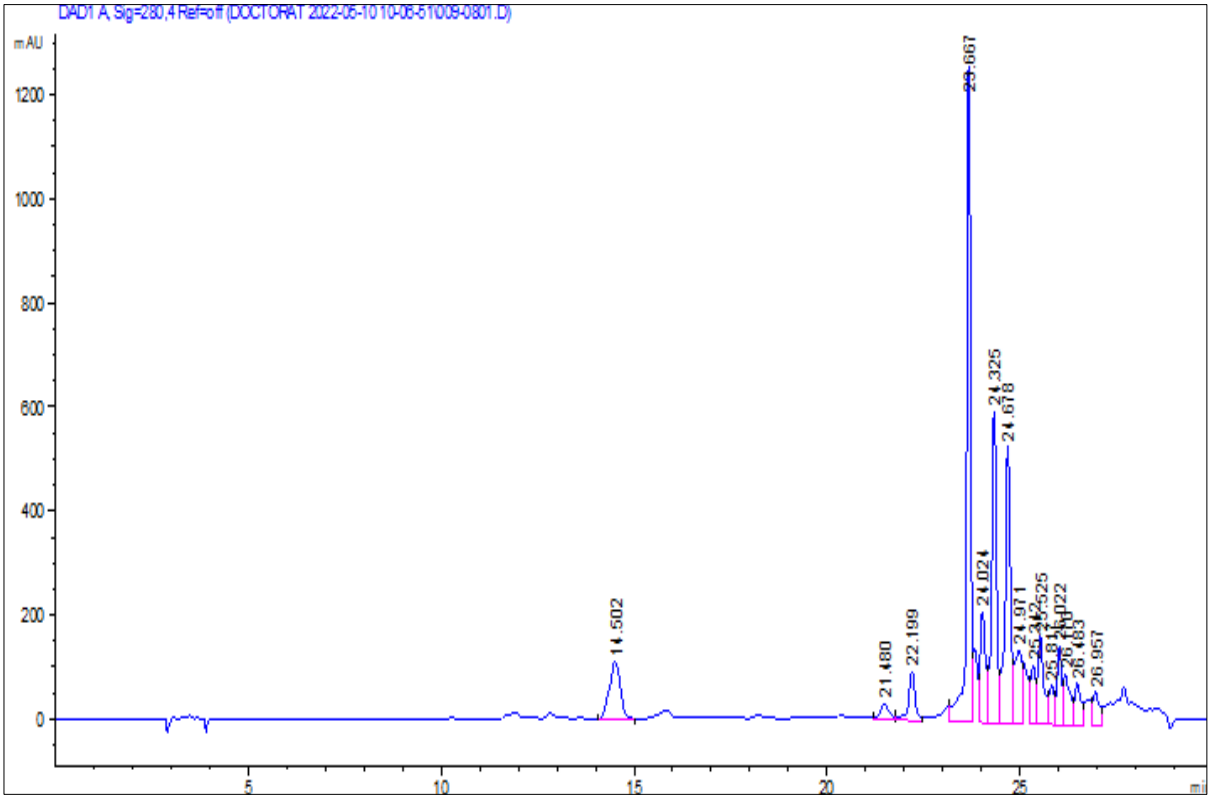

Fig 2: Chromatography of dichloromethane extract recorded at wavelength of 280 nm

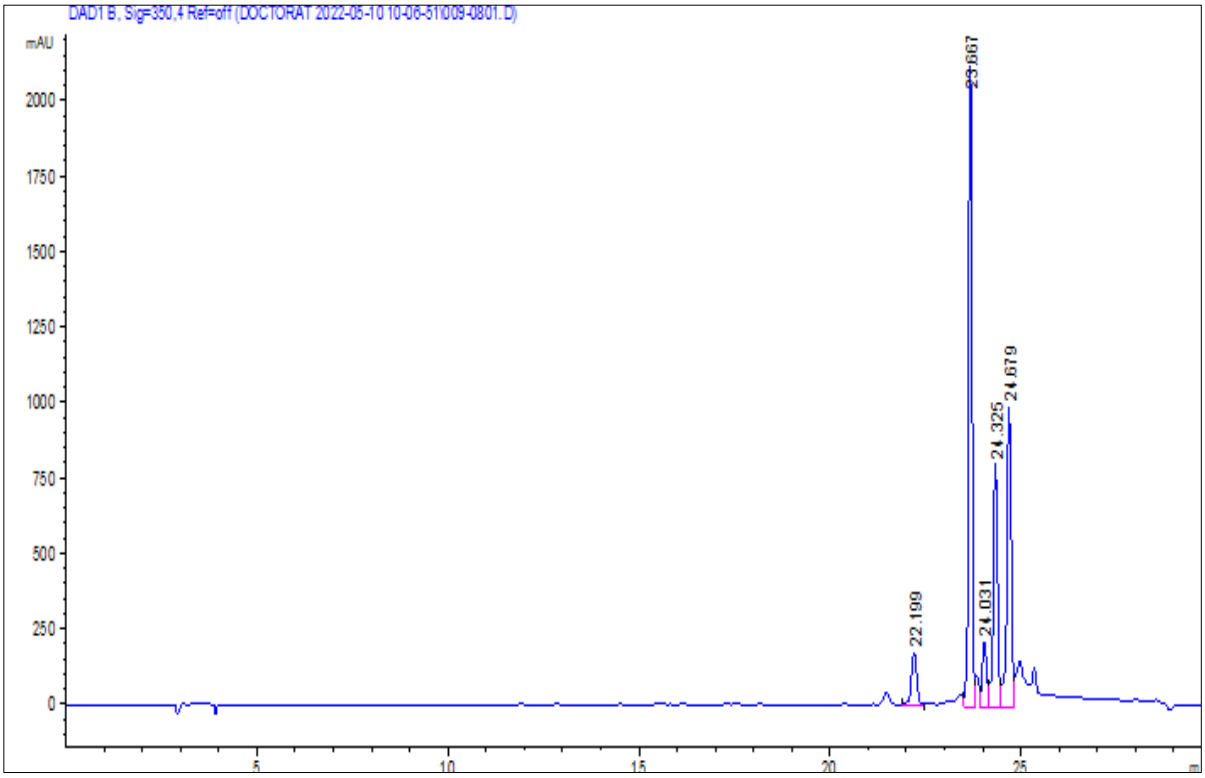

Fig 3: Chromatography of dichloromethane extract recorded at wavelength of 350 nm

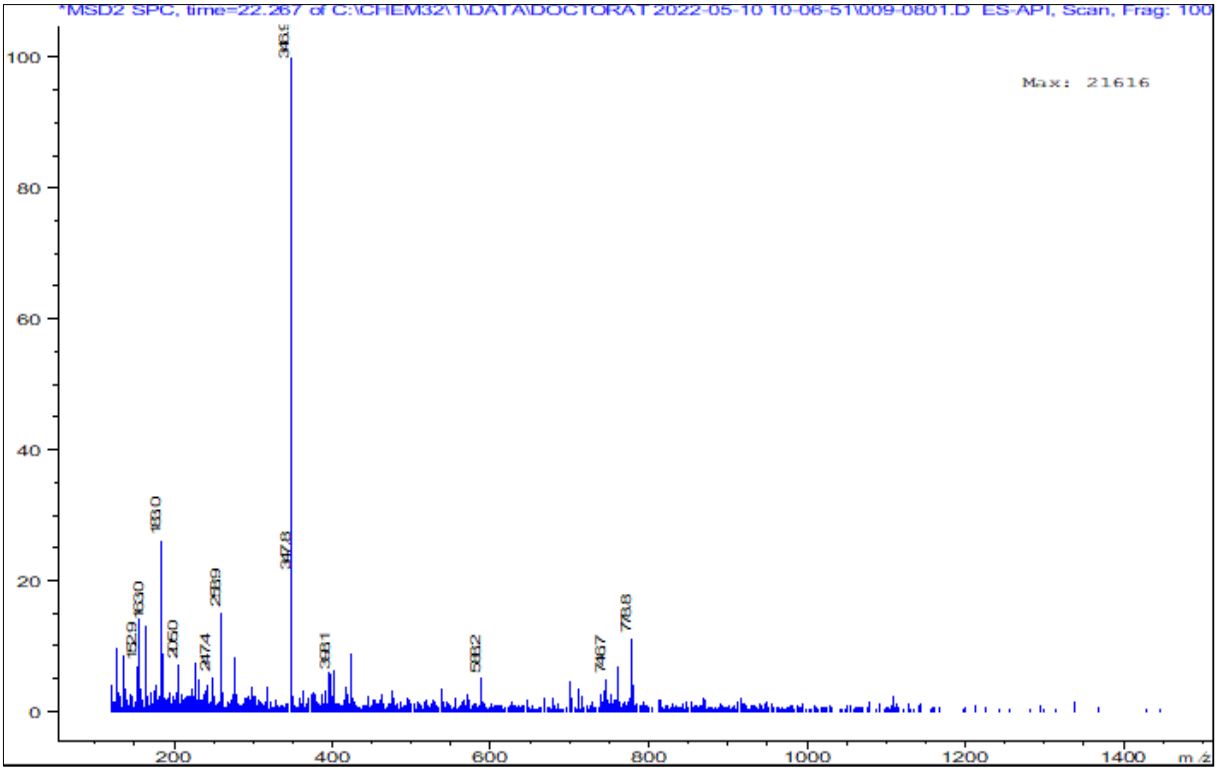

Fig 4*:* Mass spectrum of compound 1 (t_{R1} = 22.199 min)

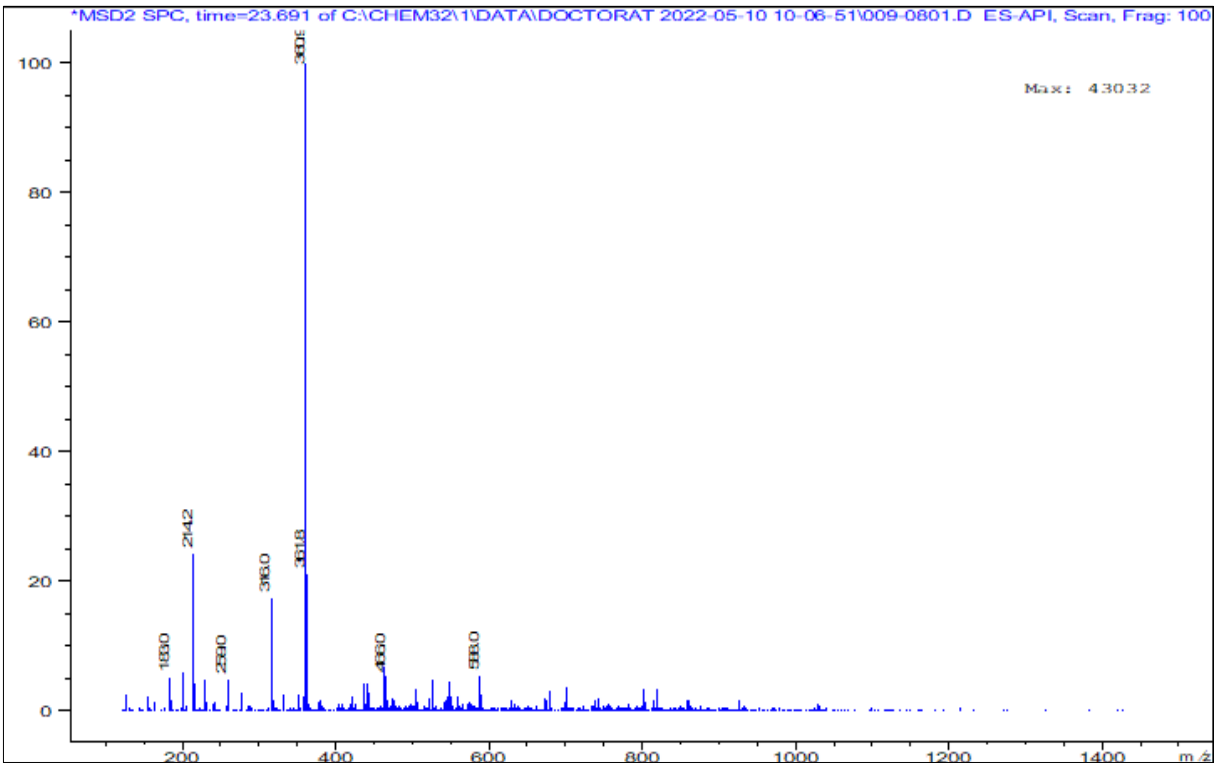

Fig 5*:* Mass spectrum of compound 2 (t_{R2} = 23.66 min)

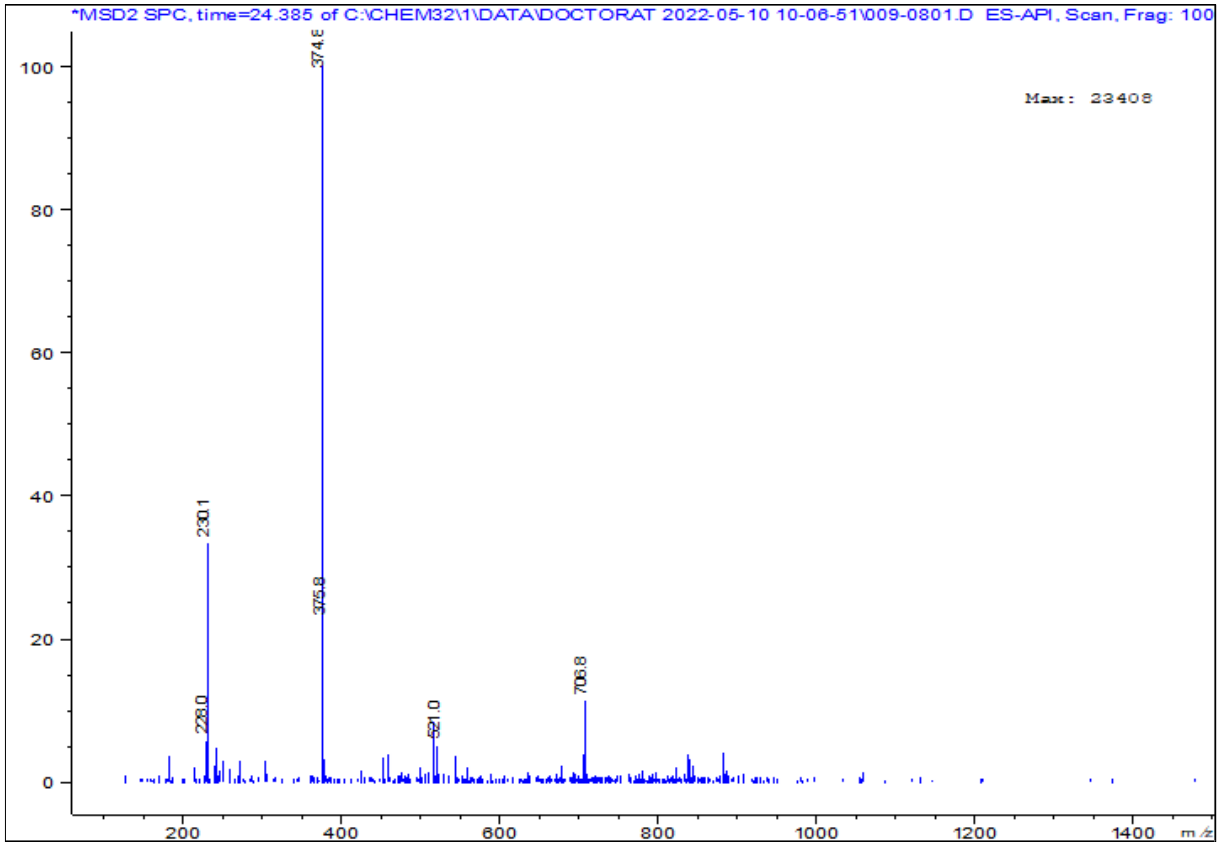

Fig 6: Mass spectrum of compound 3 (t_{R3} = 23.66 min)

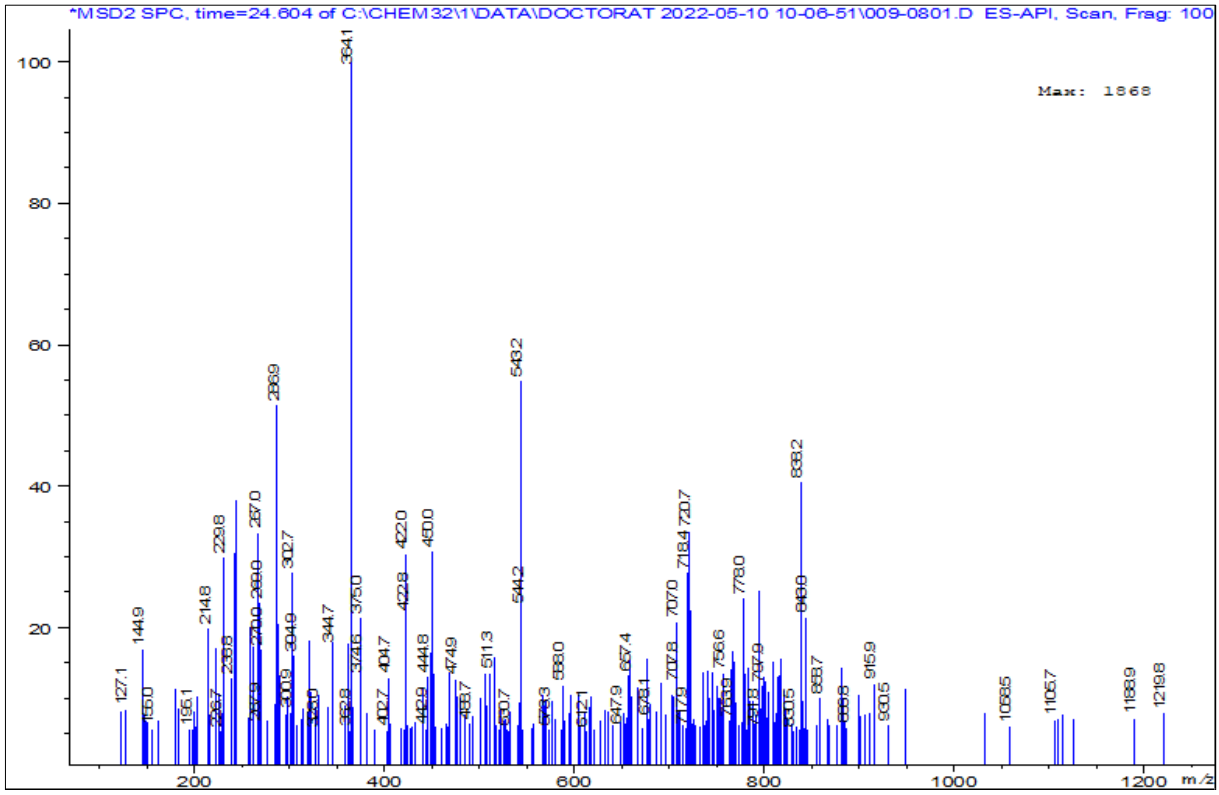

Fig 7: Mass spectrum of compound 4 (t_{R4} = 24.6 min)

6. Conclusion

This study aimed at the phytochemical characterization followed by an evaluation of the antiplasmodial activities of the solvent-based extracts of the leaves of *Acanthospermum hispidum* collected in Burkina Faso. This plant contains secondary metabolites such as flavonoids, tannins, saponins, alkaloids and carotenoids. Phenolic compounds predominate in the methanolic extract (155.79 mg GAE/g of extract) while flavonoids are more abundant in the dichloromethane extract (36.27 mg QE/g of extract).

The methanol extract and the ethyl acetate extract are also the richest in total antioxidants with respectively 271.32 and 137.98 mg TE/g of extract.

Dichloromethane and ethyl acetate extracts contain more alkaloids than the other extracts respectively at 87.43 and 124.68 µg qE/g of extract. The dichloromethane and ethyl acetate extracts showed interesting antiplasmodial activities on the sensitive strains 3D7 and D10 as well as on the resistant strain Dd2. The lowest inhibitory concentration is 2.75 µg/mL (D10) for the dichloromethane extract. The hemolysis test carried out with all the extracts revealed that the active extracts have no harmful effects on the red blood cells.

The antiplasmodial activity observed could be related to the alkaloids or flavonoids contained in the DCM extract. Dichloromethane, hexane and ethyl acetate extracts are potential antiplasmodial extracts.

Five molecules have been identified as 3, 4', 5, 7-tetrahydroxy-3',5'-dimethoxyflavone, myricetintrimethylether, 5-hydroxy-7, 3', 4', 5'-tetramethoxyflavon 3-O-rhamnoside, Quercetin 3-O-rhamnoside, and Kaempferol 3-O-glucoside.

Further investigation is necessary in order to isolate and characterize biomolecules in the active extracts with individual antiplamodial activity.

7. Acknowledgement

Authors should like to heartily thank National Malaria Research and Training Center (NMRTC) in Ouagadougou, Burkina Faso, for technical support. In the same order, we would like to thank the Higher Education Support Project (HESP) for granting us a grant to carry out our work.

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