



Original Research Article

Phytochemical screening, antioxidant and *in vivo* antiplasmodial activities of *Acacia gourmaensis* A. RICH. (Mimosaceae)

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In this present study, *in vivo* antiplasmodial and antioxidant activities of *Acacia gourmaensis* A. RICH. (Mimosaceae) were evaluated based on its total extracts (ethanol-water, decoction, acetone), fractions (hexane fraction, ethyl acetate fraction, butanol fraction and aqueous fraction) and mineral contents. Total extract of decoction, acetone and EtOH-H₂O fractions were used to evaluate chemical composition using HPLC-GC/MS-DAD methods. This plant is used in traditional medicine against liver disease, fractures, coughing fits and especially paludism. The antioxidant property was analyzed using 3 methods such as total antioxidant activity (TAC), iron reducing power (IRP) and radical scavenging activity (DPPH*). Antiplasmodial activity was performed in mice using red blood infected by *Plasmodium berghei*. The biological activities (antioxidants and antiplasmodial) were directed against plant phytochemical content 9 polyphenolic compounds were highlighted in the HPLC-DAD method, against 3 in HPLC- GC/MS. EtOH-H₂O gave the best anti-TAC with 6.53 ± 0.15 mgEAA/mL. However, this extract (EtOH-H₂O) gave the weakest activity with radical scavenging activity (23.73 ± 0.11 µg/mL). The best anti-DPPH* is obtained with acetone extract (1.93 ± 0.05 µg/mL). Concerning anti-IRP activity, hexane fraction gave the best result with 0.12 ± 0.00 µg/mL. At the antiplasmodial level, the best percentage reduction (19.9 %) is obtained with a dose of 150 mg/kg. On the phytochemistry plan, aqueous and ethyl acetate fractions given the best content with respectively 119.14 ± 8.13 mg EAG/g for total phenolic and 5.33 ± 0.17 EQ/g for total flavonoids. Mineral content quantification show that this plant is rich in calcium with 23.43 ± 1.32 mmol/L. The results due to the antiplasmodial activity could justify the traditional use of this plant.

Key words: antiplasmodial, antioxidant, phytochemistry, *Acacia gourmaensis*

INTRODUCTION

In 2016, 216 million cases of paludism was reported in 91 countries, an increase of 5 million compared to the previous year (WHO, 2017). There were an estimated 445000 deaths globally from paludism. 91% of these deaths concerned African Region, thus 80 % in sub-Saharan Africa (WHO,

2017). In Africa, millions of people still lack access to the tools they need to prevent and treat the disease (WHO, 2017). In Burkina Faso, malaria remains a public health problem, particularly during the transmission period where 60% of cases occurred in children (Tiono et al., 2014).

In the central region of Burkina Faso, aerial parts, bark or stems leaves of *Acacia gourmaensis* A. RICH. (Mimosaceae) is widely used for the treatment of malaria and another disease (Guinko 1991; Nacoulma 1996). According to Nacoulma (1996), this plant (aerial parts, bark, stems and leaves) is used in traditional medicine against liver disease, fractures, dermatosis and paludism. Nacoulma (1996) also indicated that this plant contained tannins, saponosides, terpenoids, sterols and amines.

Taking account of literature data, African patients are turned over towards traditional medicine either ignorance or for socio-economic reasons or cultural reasons (Kerharo, 1975; Beiersmann et al., 2007; Bangou et al., 2012). We want through this study to bring some scientific evidence to the traditional use of this plant. To our knowledge, we didn't meet in the literature, the antiplasmodial work on this plant. The goal of the present study was to investigate *in vivo* antiplasmodial and antioxidant against *A. gourmaensis* total extracts (ethanol-water, acetone and decoction), fractions (HF, BF, EAF and AqF) and minerals contents. More specifically we evaluated: (1) the antioxidant activity through three methods and (2) *in vivo* antiplasmodial activity on NMRI mice infected with *Plasmodium berghei* (ANKA strain) against phytochemical screening.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile (HPLC grade), water (HPLC grade), ethanol (HPLC grade), ethyl acetate (analytical grade), hexane (analytical grade), sulfuric acid (analytical grade), and sodium phosphate were purchased from J. T. Baker (Xalostoc, Mexico). 2,2-Diphenyl-1-picrylhydrazyl (DPPH*), aluminum chloride, ammonium molybdate, and the references: quercetin, quercitrin (quercetin-3-rhamnoside), caffeic acid, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis Missouri, USA). The standards kaempferol-3,7-*O*-diglucoside, quercetin-3-*O*-[rhamnosyl-(1-6)-galactoside], kaempferol-3-*O*-[rhamnosyl-(1-6)-glucoside] came from Apin Chemicals Limited (Abingdon, Oxon, UK). Trichloroacetic acid and ferric chloride were obtained from Merck (Darmstadt, Germany). Potassium ferricyanide was purchased from Fermont (Monterrey, Mexico).

Plants materials

Bark of *Acacia gourmaensis* A. RICH. was collected in Burkina Faso in October 2013 at Gampella (25 Km East from Ouagadougou). The plant was botanically identified by Professor Millogo-Rasolodimby from the Plant Biology Department of the University of Ouagadougou. The Voucher specimens was deposited in the OUA herbarium of the CIB (Centre d'Informationsur la Biodiversité), UFR/SVT of the University of Ouagadougou.

Extraction and fractionation

Polyphenols and flavonoids were extracted from dry ground (bark) by maceration (4 g) in 40 mL of: ethanol 80% (v/v) and the mark re-extract with ethanol 20% (v/v) = ethanol extract, acetone 100% and decoction, for 24 hours in darkness, and at room temperature. The extracts were centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatants separated. The pellets (only for EtOH-H₂O extract) were re-extracted in 100 mL of 20% ethanol (v/v) for 3 hours, centrifuged under the same conditions, and the supernatant decanted. Both supernatants were combined to form the total extracts, each extract was used for further analysis. Each totals extract was concentrated to dry for polyphenolic, flavonoids, antioxidants and plasmodial activities. Before an aliquot of EtOH-H₂O extract was concentrated to half the volume and then fractioned twice respectively with n-hexane, n-butanol and ethyl acetate solvents. Each fraction was individually concentrated to dryness and directed against the 2 types of biological analyses.

Parasites

Experiments were performed using *P. berghei* ANKA strain initially obtained from the Malaria Research and Reference Reagent Resource Center (MR4) and carefully maintained in our laboratory.

Experimental animals

The experiment was performed in female NMRI mice aged from 8 to 12 weeks and weighing 25±2g. Animals obtained from the International Center for Research and Development on Livestock in Sub-humid Areas (CIRDES), Bobo-Dioulasso were housed in the same environmental conditions (temperature 25°C, 12h photoperiod and fed by standard food provided by "Service Régional d'Élevage Bobo-Dioulasso", Burkina-Faso).

Antioxidant activity determination

Total Antioxidant Capacity

The total antioxidant capacity (TAC) of each sample was evaluated through the method developed by Prieto et al. (1999), in which the reduction of Mo (VI) to Mo (V) is carried out by the antioxidant, forming a green phosphate/Mo (V) complex at acidic pH. Aliquots (100 µL) of each sample (containing 100 µg.mL⁻¹ of flavonols, respective concentrations of flavonols calculated from the standard curve of quercetin) were prepared and combined with 1 mL of a solution constituted of sulfuric acid (0.6 M), sodium phosphate (28 mM), ammonium molybdate (4mM) and incubated at 95°C for 90 min. After reaching room temperature, the absorbance of each samples was registered at 695 nm against a blank prepared as indicated for the samples but adding ethanol instead of the sample.

The reference quercetin was analyzed in the same manner. TAC was expressed as mg ascorbic acid equivalents. Ascorbic acid curve: $A_{695} = 3.678x - 0.092[\text{ascorbic acid}]$, correlation coefficient $R^2 = 0.998$, constructed with ascorbic acid between 1.0 and 30.0 mg.mL⁻¹. The analysis was done for independence aliquots of the samples from three pools of samples.

Iron Reducing Power

The iron reducing power (IRP) method reported by Yang, Guo and Yuan (2008) was used to evaluate the iron reducing power of each simple. Aliquots (1 mL) of each sample were combined with 2.5 mL (phosphate buffer, 0.2 M, pH 6.6), 2.5 mL (potassium ferricyanide, 30mM) and incubated at 50 °C for 20 min. After, 2.5 mL trichloroacetic acid (0.6 M) was added and the mixture was centrifuged (2000 rpm for 10 min). From the upper layer, 2.5 mL of solution was removed and distilled water (2.5 mL) and ferric chloride (0.5 mL, 6 mM) were added to it. The absorbance at 700 nm of the formation of ferrous ions (Fe²⁺) was registered after 10 min. The highest absorbance values indicated the greatest capacity of reducing ferric (Fe³⁺) to ferrous (Fe²⁺) ions. Four flavonol concentrations (10-400 µL combined with the proper volume of ethanol to reach 1 mL as final volume) of each sample (respective concentrations of flavonols calculated from standard curve of quercetin) were evaluated. The reducing power was expressed in terms of EC₅₀ (mg.mL⁻¹). The evaluation was separately done for the samples from three pools of samples.

Free radical scavenging activity

The DPPH* method reported by Campos et al., (1994) was used to evaluate the free radical scavenging activity. Four to five flavonoid concentrations of each sample were individually added to a DPPH* solution (40 µg.mL⁻¹ in ethanol) in such a way so as to maintain a final volume of 1 mL. The decrease in absorbance was determined at 523 nm after 10 min. The DPPH* concentrations of samples were plotted to determine by linear regression, the efficient concentration at 50 %, defined as the amount of antioxidant needed to decrease by 50 % the initial DPPH* concentration (EC₅₀). The following calibration curve, made with DPPH* between 6.25 and 100 µg.mL⁻¹, was used to calculate the DPPH* concentration (µg.mL⁻¹) in the reaction medium: $A_{523} = 0,030x + 0,001 [\text{DPPH*}]$, correlation coefficient $R^2 = 0,999$. Antioxidant activities were expressed in terms of EC₅₀ in µg.mL⁻¹. The analysis was separately done for the samples from three pools samples.

Antiplasmodial activity determination

In vivo antiplasmodial activity test

Experiments were performed in mice according to the 4 days suppressive test (Peters and Robinson 1992). The

mice were divided in four groups of 6. At day 0, mice were inoculated intraperitoneally with 10⁷ red blood cells infected by *Plasmodium berghei* (ANKA stain). Two hours post infection, the animals received orally 200µl of three doses of extracts 100, 250 and 500 mg/kg bw, once a day from day 0 to day 4. The control group received only the solution used to dissolve the extracts. On day 4 post infection, blood smears obtained from the tail of the mice were fixed with methanol, stained with Giemsa 10% and read with a microscope, under a 100x oil immersion objective. Mean parasitemia from each group was recorded and the percentage inhibition of parasitemia calculated according to the formula described by Fidock et al. (2004)

$$\% \text{ inhibition} = \frac{\text{Control group parasitemia} - \text{Tested group parasitemia}}{(\text{Control group parasitemia})} \times 100$$

Phytochemical analysis

Determination of total phenolics, total flavonoids and minerals

Total phenolics

Folin-Ciocalteu method was used for measurement of total content of phenolic compounds according to Nurmi et al. (1996) method, by linear regression analysis from the standard curve of gallic acid ($Y = 0.003x + 0.016$; $R^2 = 0.997$). A 250 µl extract was mixed with 2.5 ml of desionized water. Afterwards, 125 µL Folin-Ciocalteu reagent was added and the mixture was allowed to stand for 5 min. Finally, 375 µL of 20% Na₂CO₃ was added. After 2 hours incubation at room temperature, the absorbance was measured at 760 nm on a Spectroscopic Analysis Mecasys (Optizen). Three replicates of each sample were analyzed.

Total flavonoids

Flavonoid content was determined according to Lauranson-Broyer and Lebreton (1993) by linear regression analysis from the following standard curve of quercetin: $Ab_{S425nm} = 0.025x + 0.014 [\text{Quercetin}]$, correlation coefficient $R^2 = 0.998$. The curve was registered after the addition of 60 µL of a freshly prepared 5% (w/v) aluminum chloride solution to 1 mL of quercetin solution (four different concentrations in the range of 100 to 1400 µg/mL). The absorbance was immediately registered after the addition of aluminum chloride, at 425 nm, using a Spectronic Genesys 2 spectrophotometer (Rochester, New York, USA). The flavonoid content in each sample was also registered after the addition of aluminum chloride and expressed as µg of quercetin equivalents/ g dry extract. The addition of aluminum chloride produces bathochromic shifts (which can be perceived by a yellow coloration) in flavonoids containing orthodihydroxyl groups, due to the formation of complexes between the aluminium and C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and

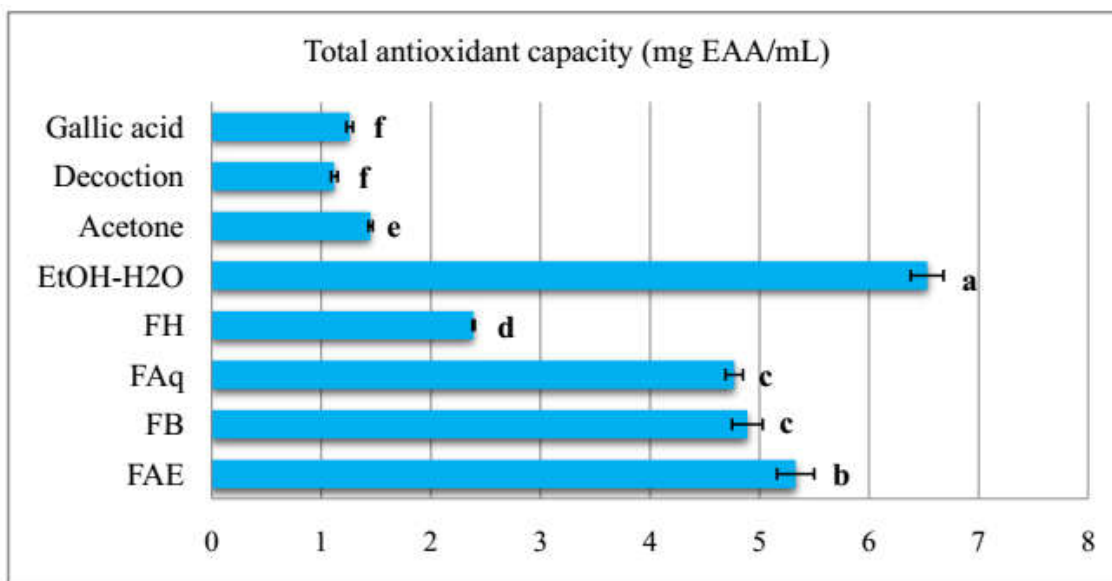


Figure 1: Total antioxidant capacity (mgEAA/mL)

flavonols; complexes also are formed between the aluminum and the orthodihydroxyl groups in A or B-ring of flavonoids (Mabry et al., 1970). The addition of aluminium chloride represents a standard procedure for reproducibility (Lauranson-Broyer and Lebreton, 1993). The determination of flavonoid content was estimated individually for three pools of samples.

HPLC-MS/GC and HPLC-DAD analysis

In this level, two chromatographic methods were used to determine secondary metabolites (Mabry, Markham and Thomas, 1970; Campos and Markham, 2007; Bangou et al., 2017): (1) HPLC-DAD and (2) HPLC-MS/GC. Concerning the second method, ethanol-water fraction was used. The extract was concentrated to dryness. An aliquot of extract was re-dissolved with some drops of ethanol-water, and the volume was supplemented to 5 mL of acetone 100%. The extract was centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatants separated. 1 mL of supernatant was taking for the HPLC-MS/GC (Clarus 500 Perkin Elmer) analysis using flame ionization detector (FID). Injection volume (0.5 μ L), column (Elite 5), carrier gas (He), recording time lies between 2-22 mn and the temperature of ionization lies between 60 to 220 $^{\circ}$ C. Structural identification was obtained by direct comparisons of retention times and INST1 spectra of resolved compounds.

Atomic absorption (Samples preparation)

Typical analytical procedure of 975.03 to AOAC was used adapted by Perkin-Elmer (1982). 1 g of ground dried plant sample should be taken and placed in a small beaker. 10 mL

of concentrated HNO_3 should be added and allowed to stand overnight. It should be Heated carefully on a hot plate until the production of red NO_2 fumes has ceased. The beaker should be cooled and small amount (2 – 4 mL) of 70 % HClO_4 should be added. It should be Heat again and allowed to evaporate to small volume. The sample should be transferred to 50 mL the volume should be flaked and diluted with distilled water. Caution: HNO_3 should always be added to the tissue sample and the mixture should be digested before adding HClO_4 . HClO_4 can react explosively with untreated organic matter.

Statistical analysis

Statistical analysis: All assays were carried out in triplicates and results are expressed as Means \pm Standard Deviation (SD) calculated with Excel 2007. Statistical comparisons were done with the XLSTAT7.5.2, using Spearman correlation. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidants investigations

Total antioxidant capacity

The results of this study are ranged between 1.12 ± 0.03 to 6.53 ± 0.15 mg EAA/mL (Figure 1). Ethanol-water extract (6.53 ± 0.15 mg EAA/mL) had the highest total antioxidant capacity. Following by that of ethyl acetate fraction (5.35 ± 0.10 mg EAA/mL) > butanolic fraction (4.89 ± 0.14 mg EAA/mL) and aqueous fraction (4.77 ± 0.08 mg EAA/mL)

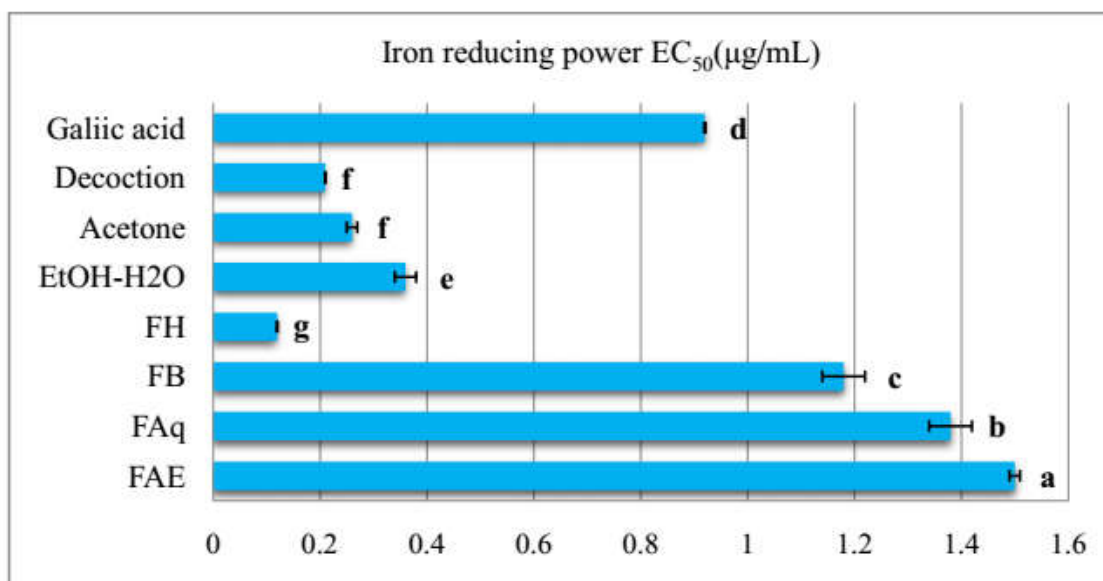


Figure 2: Iron reducing power EC₅₀(µg/mL)

(Figure 1). All the extracts gave an activity \geq to that of the reference. There isn't a very great difference between the results of the fractions. Indeed, they line up between 2.39 ± 0.01 and 5.33 ± 0.17 mg EAA/mL. However, the results of ethanol-water (6.53 ± 0.15 mg EAA/mL) is approximately 6 times superior with those of the acetone (1.45 ± 0.02 mg EAA/mL) and the decoction (1.12 ± 0.03 mg EAA/mL).

Among the *Acacia* genus, *nilotica* species would be most studied (Guinko, 1991; Nacoulma, 1996; Tahir et al., 1999; Atif et al., 2012; Benbrahim et al., 2014; Kutama et al., 2018). To our knowledge, there wouldn't have to meet study on the antioxidant properties of *A. gourmaensis*. But total antioxidant capacity could be explained by the phytochemical content such as polyphenol and flavonoids contain. Indeed, it has been recognized that polyphenol and flavonoids contents of plants had a good antioxidant candidate (Nacoulma, 1996; Proestos et al., 2006).

Iron reducing power

IRP method allows to measure the capacity of the phenolic to reduce the ions Fe (III) to Fe (II). The ascorbic acid was used as standard for the development of the calibration curve whose regression equation is: $y = 2.6486x + 0.0017$; $r = 0.9988$. The results of this study are indicated in Figure 2. Gallic acid was used as reference (0.92 ± 0.001) at a same concentration (100 µg/mL). The Iron reduction power of sample was expressed as EC₅₀ value. Globally the hexane fraction and the total extract: EtOH-H₂O, acetone and decoction exhibited a good iron reducing power with respectively 0.12 ± 0.00 µg/mL (HF) $> 0.21 \pm 0.00$ µg/mL (decoction) $> 0.26 \pm 0.01$ µg/mL (acetone) and 0.36 ± 0.02 µg/mL (EtOH-H₂O). Also, in this level our results with *Acacia gourmaensis* (Mimosaceae) are very high compared

to those of Bangou et al., 2017 (Verbenaceae).

Radical DPPH

The antiradical activity of samples was expressed as EC₅₀ value, which represented the effective concentration of sample required to scavenge 50% of radical's DPPH. This assay was used to evaluate the antioxidant activity of *A. gourmaensis* extracts. At this level the results varied 1.93 ± 0.05 to 23.73 ± 0.11 µg/mL. Acetone total extract had a highest EC₅₀ value (1.93 ± 0.05 µg/mL), following by decoction $>$ ethyl acetate fraction $>$ hexane fraction respectively with EC₅₀ value of 3.98 ± 0.01 ; 5.81 ± 0.22 ; 7.13 ± 0.06 (Figure 3). By comparing our results (low value: 23.73 ± 0.11 µg/mL) with those of Bangou and collaborators (2017) who used the same method (low value: 13.93 ± 0.39 µg/mL), we note that our results are very high. These last authors worked on 4 species of Verbenaceae family with the same types of extractions. In terms of radical scavenging activity, their best result was obtained with the *L. chevalieri* (EAF = 0.05 ± 0.00 µg/mL) species. We can conclude that these authors had better results on the 4 species of plants (Verbenaceae) compared to *Acacia gourmaensis* which is of the Mimosaceae family.

Plasmodial investigations

In vivo antiplasmodial activity

Vis-a-vis the emergence and with the resistance of a new form of paludism vector, it's necessary to seek biological molecules able to counter these parasites. To this end, we evaluated the *in vivo* antiplasmodial activity of EtOH-H₂O extracts of *Acacia gourmaensis* on the old mice from 8 to 12

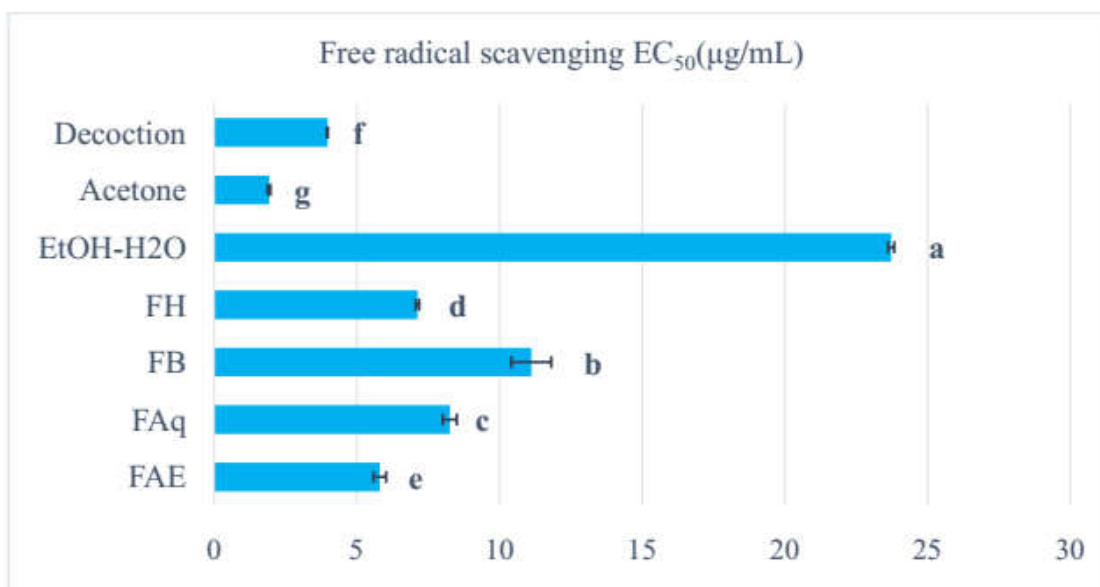


Figure 3: Free radical scavenging EC₅₀(µg/mL)

Table 1. Parasites inhibition by EtOH-H₂O extracts of *Acacia gourmaensis*

burk extracts	Yield (%)	Dose (mg/kg) per group	Parasitemia (CI 95%)	% Reduction
EtOH/H ₂ O	11.38	Control group	35.3±0.5	-
		100	33.0±1.2	6.4
		150	28.3±4.6	19.9
		200	28.5±5.6	19.1
		250	29.8±1.6	15.6

weeks. The results are contained in Table 1. With 150 mg/kg we reached 19.9 % of *Plasmodium berghei* reduction. According to Rasoanaivo et al. 2004, an *in vivo* antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a percent parasite suppression equal to or greater than 10%, 50%, 90% at a dose of 250 mg/kg body weight per day, respectively (Rasoanaivo et al., 2004). By comparing our results with those of the classification, the antiplasmodial activity of EtOH-H₂O extracts of *Acacia gourmaensis*, were found to be moderate (19.9%) of inhibition dosed of 150mg/kg. Several studies showed that this antiplasmodial activity is correlated with secondary metabolites contents such as polyphenols and flavonoids (Khalid et al., 1986; Cubukcu et al., 1990; Tahir et al., 1999; Bagavan et al., 2011; Adia et al., 2016). In 1999, Tahir and collaborators had accused terpenoids and tannins in the best antiplasmodial activities observed against *P. falciparum* of the extracts of *A. nilotica*. The highest activity on *P. falciparum* was IC₅₀ = 1.7 mg/mL. Certain polyphenolic compounds such as quinoline alkaloids isolated from plants are active against *Plasmodium falciparum*. *Acacia nilotica* of the same family showed itself to be antiplasmodial. Also, the overexpression

of reactive oxygen species causes a malfunction of the body that can cause the occurrence of malaria (Bangou et al., 2017). So you need a balance between oxidizing and antioxidants.

Phytochemical investigations

Ions quantification

Eight minerals (Ca²⁺, Fe²⁺, K⁺, Mg²⁺, Na⁺, Zn²⁺, Pb and As) were analyzed according to the method described of Perkin-Elmer (1982). In terms of ions quantification, the values are varied between 3.7x10⁻³ and 46.95 g/kg of dry weight. Calcium presented the best contents with 46.95 ± 2.65 g/kg > 4.74 ± 0.16 g/kg (Potassium) > 4.17 ± 0.12 g/kg for Magnesium (Table 2). Our results are comparable to those of Bangou et al. (2017) which also found Calcium (55.88 ± 1.38 g/kg) with the best value. In a general way calcium had been the strongest value among the 4 plant species studied by Bangou et al. (2017). We can note in terms of minerals and polyphenolic compounds content that *A. gourmaensis* (Mimosaceae) and the 4 Verbenaceae had similar contained. It's known that the care by traditional medicine is generally related to the

Table 2. Quantitative analysis of minerals

Elements contents	g/kg	mmol/L
Ca ²⁺	46.95 ± 2.65	23.43 ± 1.32
Mg ²⁺	4.17 ± 0.12	3.43 ± 0.10
K ⁺	4.74 ± 0.16	2.43 ± 0.08
Na ⁺	0.0424 ± 0.001	0.037 ± 0.001
Fe ²⁺	0.204 ± 0.005	0.073 ± 0.002
Zn ²⁺	0.0037 ± 0.0004	0.0011 ± 0.0001
Pb	< 0.000005	< 0.000005
As	< 0.000005	< 0.000005

combinations of plants, fragment of animals and minerals. In a general way the mineral absences involves disturbances (Sawadogo et al., 2015; Bangou et al., 2017). Human body needed daily for the ions 2g/day (sodium) > 1.5 g/day (magnesium) and of potassium (0.5 g/day). Magnesium deficiency can involve syndromes of neuromuscular hyperirritability, cardiovascular and thrombosis accidents. Its metabolism is also related to those of Ca²⁺ and P (Nacoulma, 1996).

Total phenolic and flavonoid

The analysis of the totals phenolic and flavonoids content varied respectively from 5.66 ± 0.99 to 119.14 ± 8.13mg EAG/g dry extracts and 0.24 ± 0.02 to 5.33 ± 0.17 mg EQ/g dry extracts. The highest contents are held by the fractions in order 119.14 ± 8.13 (AqF) > 52.03 ± 5.54 (EAF) > 26.69 ± 0.71 (BF) for the phenolic one and in order 5.33 ± 0.17 (EAF) > 3.57 ± 0.32 (AqF) > 3.01 ± 0.41 (BF) for the flavonoids. Then, total extracts come in order of EtOH-H₂O (27.81 ± 2.59: total phenolic and 2.05 ± 0.05: total flavonoids) > acetone (19.09 ± 0.02: total phenolic and 1.02 ± 0.04: total flavonoids) > decoction (5.66 ± 0.96: total phenolic and 0.24 ± 0.01: total flavonoids) Figures 4& 5. By comparing our quantification results with those of Bangou et al. (2017), we note that our results are in the same order (fraction before total extracts contents). While the antioxidants activities showed that the Verbenaceae family was better than that of Mimosaceae; the concentration and the variation in the content of the various types of extracts are nearly identical.

HPLCs analysis

HPLC-DAD method applied in decoction extract and EtOH-H₂O fractions enable us to highlight 9 compounds (Table 3 and Figure 6). HPLC-GC/MS method applied to the same fractions enabled us to highlight 3 compounds (Table 4 and figure 7). Secondary metabolites highlighted on this level could explain the various activities of *A. gourmaensis* observed especially in antiplasmodial level. Former investigations showed that the flavonoids are good antioxidant candidates and more precisely those polyhydroxylated such as the derivative of luteolin (Tsimogiannis and Oreopoulou, 2004). The HPLC-GC/MS

analysis enabled us to highlight terpenes which will justify in our case, the antioxidant activities (Table 4). Other authors showed that rosmarinic acid (Penchev et al., 2010) are antioxidant compounds. Mevy et al. (2007) reported that β-caryophyllen, 1,8-cineol and germacren are antioxidant compounds (Bangou et al., 2012). With through thus this information: Which relations there are between these various compounds and the biological activities?

Relationship between biological activities and phytochemicals composition

We have evaluated the correlations between polyphenolic compounds (phenolic and flavonoids) and antioxidant activities to appreciate secondary metabolites implication. Table 5 indicates the relationship between biological activity and phytochemical investigation. These correlations varied from 0.0014 (anti-DPPH*) to 0.6367 (anti iron reducing power). The best correlations was observed between IRP (R² = 0.6367) and total antioxidant capacity (R² = 0.5325), and that of total flavonoids content. There is a very low activity between radical scavenging activity and totals phenolic (R² = 0.0014) and flavonoids (R² = 0.014) contents. The best anti-DPPH* activity was given by acetone extract (1.93 ± 0.05 µg/mL). But this extract has the lowest content in total phenolic (19.09 ± 0.02 mg EAG/g dry extracts) and total flavonoids (1.02 ± 0.04 mg EQ/g dry extracts). We can thus think that acetone extracts contained the essential ones from of the anti-DPPH* compounds. That seems to be confirmed on the level of the transformation of the Fe³⁺ into Fe²⁺. Indeed, the best activity is held by acetone extract (0.26 ± 0.01 µg/mL) and particularly decoction extract (0.21 ± 0.00 µg/mL). It would seem that these activities are influenced by the total flavonoid's contents, testifies the values of its correlations (R² = 0.6367: IRP and R² = 0.5325: TAC). Ethanol-water extract gave the best activity anti total antioxidant capacity (6.53 ± 0.15 mg EAA/mL). In this level acetone (1.45 ± 0.02 mg EAA/mL) and decoction (1.12 ± 0.03 mg EAA/mL) extracts gave the low values.

However, the correlation between polyphenolic compounds and antioxidant activities always raised their difficulties. Because sometimes it was observed a correlation, sometimes not. According to the former investigation, it was difficult to establish a clear correlation between the flavonoids rate and the DPPH* reduction

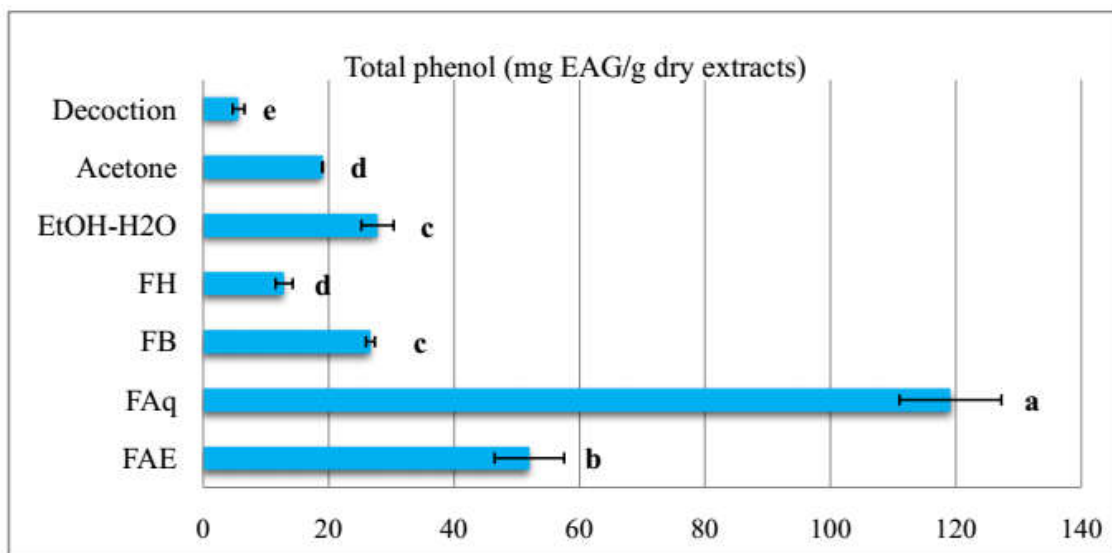


Figure 4: Total phenol (mg EAG/g dryextracts)

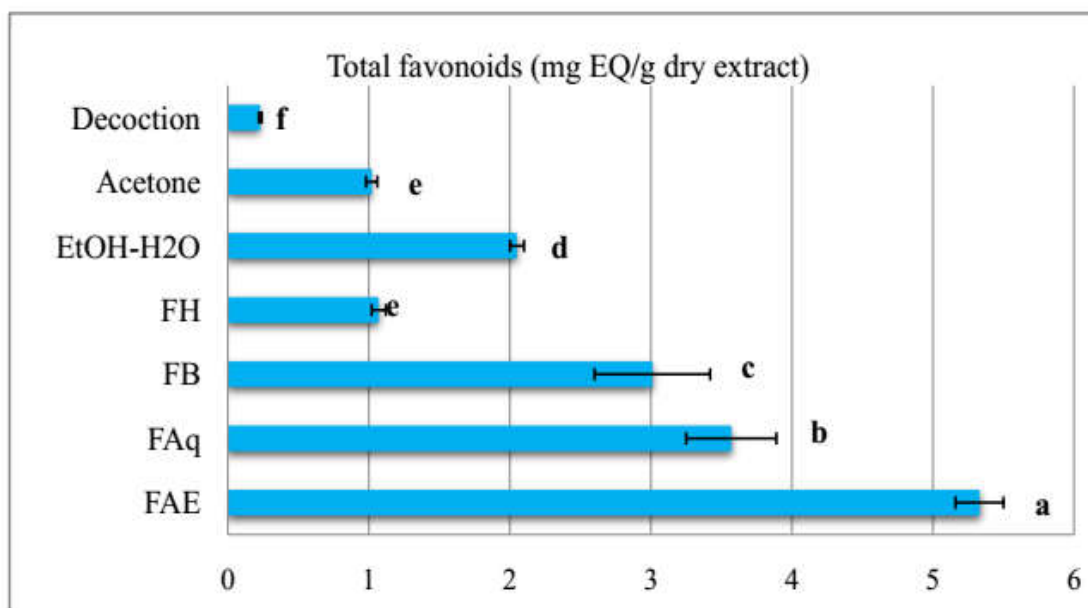


Figure 5: Total flavonoids (mg EQ/gdry extract)

Table 3. Wavelength characteristic of the phenols acids and flavonoids detected

Number of compound	Compound	RT (min)	λ_{max} (nm)
Dcago1	Scutellarein-6,4'-dimethylether	53.581	274, 331
Dcago2	Flavone	55.576	269sh, 280sh, 326
Dcago3	Flavone	57.218	262sh, 272, 285, 302sh, 317
Dcago4	Derivative of baicalein	57.438	274sh, 285, 323
Dcago5	Derivatetricin-5-O-glicoside	62.234	266, 292sh, 346
Dcago6	Rosmarinic acid	62.466	250sh, 290, 320
EcagoEAF1	Derivative of tricin-5-O-glicoside	47.333	267, 295sh, 350
EcagoEAF2	Derivative of luteolin	47.465	252, 267, 290sh, 348
EcagoHF1	Chlorogenic acid	59.492	245sh, 296sh, 323

D: decoction, E: ethanol-water, EAF: ethyl acetate fraction, HF: hexane fraction, CAGO: *A. gourmaensis*

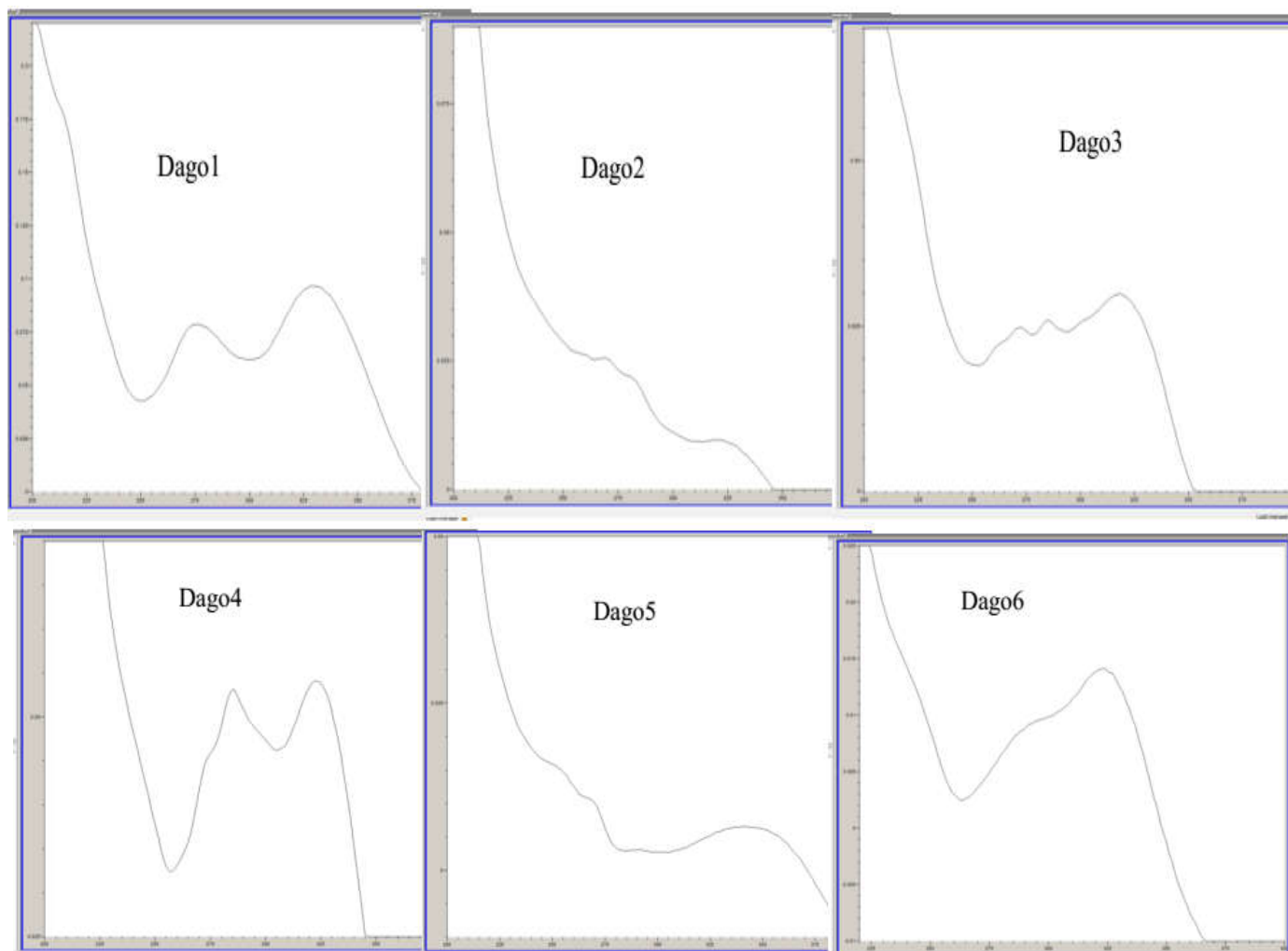


Figure 6 A: UV spectra of 9 phenols acids flavonoids found in *A. gourmaensis* (1/2). Dago: decoction extract of *A. gourmaensis*

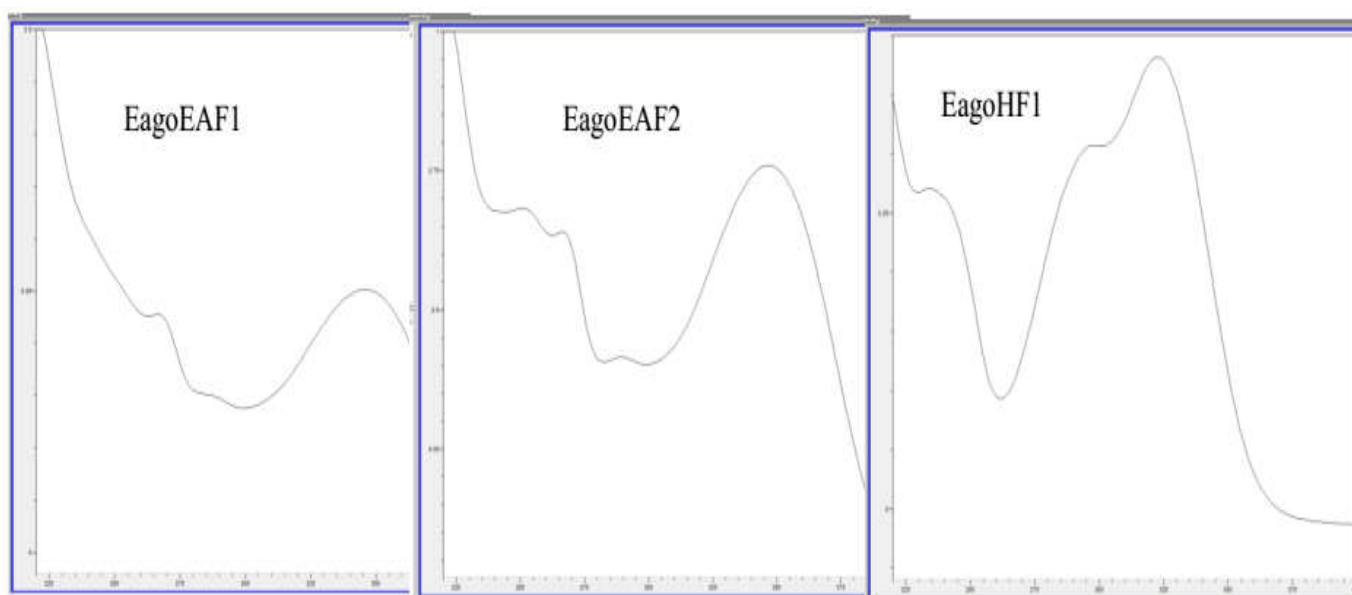


Figure 6 B: UV spectra of 9 phenols acids flavonoids found in *A. gourmaensis* (2/2). Eago: ethanol-water total extract of *A. gourmaensis*, EAF: ethyl acetate fraction, HF: hexane fraction.

Table 4. Retention time of the essential's oils detected

Species	Number of compound	Retention time (RT)	Compounds
<i>L. camara</i> ,	C1	4.439	4-Hydroxy-4-methylpentan-2-one
	C2	11.575	Di-N-octylphthalat
	C3	12.735	Fluorene

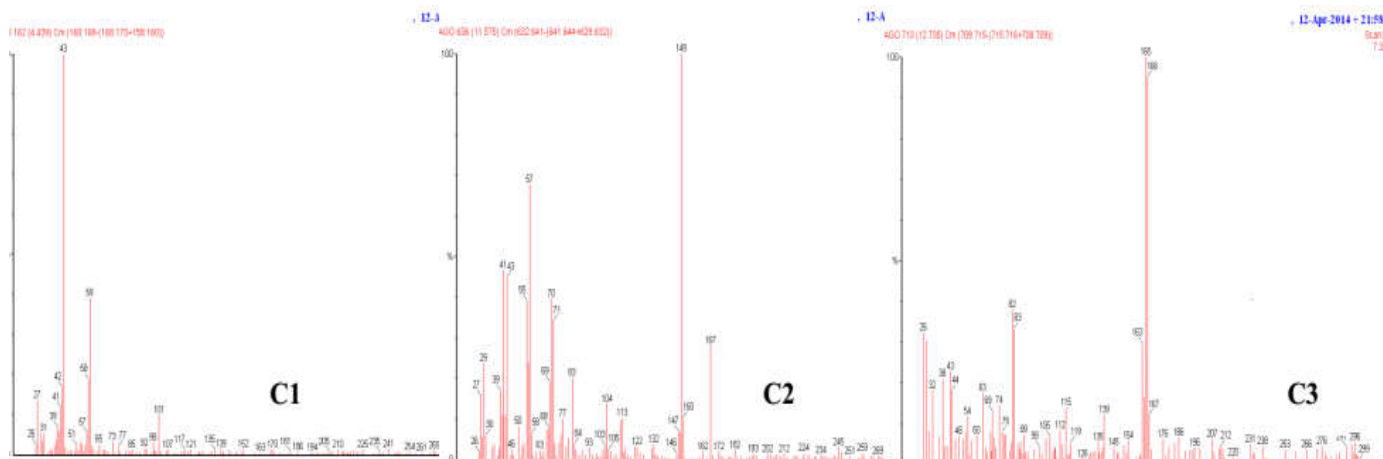


Figure 7: Spectrum of essentials oils detected

Table 5. Relationship between antioxidant activities, total phenolic and flavonoids content

R ²	TAC	IRP	DPPH*
Total phenolic	0.2344	0.362	0.0014
Total flavonoids	0.5325	0.6367	0.0134

R²: correlation coefficient, TAC: total antioxidant capacity, IRP; iron reducing power, DPPH*: radical scavenging activity

(Bangou et al., 2012). Our results are in disagreement with those of Bangou et al. (2017), but similar to those of Meda et al. (2013). The last authors were showed that antioxidant activity was correlated with total flavonoids content. Tahir and collaborators (1999) who studied the antiplasmodial activity of *Acacia nilotica* extracts concluded that the activity was correlated by the tannins and terpenoids contents. The plasmodial activity observed could be due to these compounds more especially as Nacoulma (1996) indicated that *Acacia gourmaensis* contained its.

CONCLUSION

It arises from the literature little information on *A. gourmaensis* species. The indications which exist are not thorough. In this study, we didn't meet any phytochemical investigation on this plant. However, general information on order shows that the species is used in traditional medicine. Our study shows that the EtOH-H₂O extract has a moderated activity on *Plasmodium berghei* of 19 % to 150 g/kg. In a general way, the decoction and acetone extracts were better on radical scavenging activity and anti-iron

reducing power. Also, the EtOH-H₂O extract was better on total antioxidant capacity activity. What corroborates with the traditional uses (decoction and ethanol). Other species of *Acacia* whose antiplasmodial activities were shown make the case of the implication of tannins and terpenoids. Our next studies on this plant will aim at making a bio-guided evaluation to identify the polyphenolic compounds implied on the one hand antiplasmodial activities, and on the other hand those implied in that of antioxidant.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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