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Research Article

## Pharmacological study of trunk bark of *Acacia nilotica* var *adansonii* (Guill et Perr).o Ktze (Mimosaceae): Assays, antioxidant and antispasmodic activities

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### ABSTRACT

Aim of this study was to evaluate *in vitro* polyphenols content, antioxidant and antispasmodic properties of the aqueous extract and fractions of the trunk bark of *Acacia nilotica*. According to a survey conducted in rural Burkina Faso, *Acacia nilotica* var. *adansonii* (Guill and Perr). Ktze reported to be widely used in the treatment of gastrointestinal diarrhoea and parasitosis. A maceration of the powder of the trunk bark of the plant was carried out. Then the aqueous macerate obtain, was fractionated with dichloromethane, butanol and ethyl acetate successively. The phenolic compounds of the aqueous extract, butanol and ethyl acetate fractions was determined. The antioxidant activity of aqueous extract and fractions was evaluated by the DPPH, ABTS and FRAP tests. The contractility test on smooth muscle was realized according to Magnus method. Assay of the extracts revealed a high content of polyphenols, tannins and flavonoids. The aqueous extract, the butanol fraction and the ethyl acetate fraction demonstrated a high antioxidant capacity. Aqueous extract showed a better antispasmodic effect of acetylcholine contraction induction at 1  $\mu$ M ( $IC_{50} = 13.02 \mu$ g / mL) and for BaCl<sub>2</sub> at 160  $\mu$ g / mL ( $IC_{50} = 117.2 \mu$ g / mL). The aqueous extract of *Acacia nilotica* and his fractions had antioxidant properties. Only aqueous extract proven better antispasmodic property. Hence its use in traditional medicine in the treatment of diarrhoea.

**Keywords:** *Acacia nilotica*, Antioxidant, Antispasmodic

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### INTRODUCTION

The use of medicinal plants for the treatment of various diseases is the speciality of populations in developing countries such as Burkina Faso. Four hundred and twenty-seven (427) species of plants are being used for the treatment of various diseases including gastrointestinal parasitosis in Burkina Faso<sup>1</sup>. In the search for new bioactive molecules against gastrointestinal parasitosis and diarrhoea, many researchs have been realize on the anthelmintic properties of certain plants. *Acacia nilotica* var *adansonii* (Guill and Perr) .O Ktze (Mimosaceae) plant, used in traditional medicine in the treatment of intestinal parasitosis and diarrhoea. It barks

and pods used in rural areas in the treatment of diarrhoea, verminosis and gastritis<sup>2</sup>. Some of these antiparasitic properties including anthelmintic<sup>3</sup> and antibacterial<sup>4</sup> have already been proven. The objective of this study was to determined polyphenols content and evaluated antispasmodic and antioxidant properties of the aqueous macerate of the trunk bark of *Acacia nilotica* var *adansonii*. Antioxidant activity evaluated by DPPH, ABTS and FRAPS tests. The study of smooth muscle contractility properties of the aqueous extract and its fractions were demonstrated by Magnus method <sup>5</sup>.

## MATERIALS AND METHODS

### Plant and preparation of fractions

The *Acacia nilotica* var *adansonii* plant harvested at 140km from Ouagadougou in the Central-East region where the species is highly represented<sup>6</sup>. It has been identified under number HNBU00210 at the Herbarium of the National Center for Scientific and Technological Research (CNRST). The study extract prepared according to the traditional use. It aqueous maceration adopted for the extraction. One part of this extract has been fractionated. A test sample of 200 g of the plant material (bark powder) was macerated for 24 hours. The macerate obtained, was concentrated and fractionated by the liquid/liquid method. Fractionation of the aqueous extract used successively, dichloromethane solvents, ethyl acetate and butanol. The different fractions obtained were condensed in a rotavapor and then oven-dried.

### Chemical reagents

Tyrod solution composition was KCl (0.2g), NaCl (8g), MgCl<sub>2</sub> (0.01g), NaHCO<sub>3</sub> (1g), NaH<sub>2</sub>PO<sub>4</sub> (0.05g), Glucose (1g) and CaCl<sub>2</sub> (0.2g) in 1L of water distilled. It used as a survival medium for the isolated gut flap and atropine, papaverine as references. Reagents as well as DPPH (2,2-diphenylpicrylhydrazine), ABTS [2,2'-azinobis (3-ethyl benzoin-6-sulphonate)], Trolox, quercetin, tannic acid, FCR 2N (Folin Ciocalteu reagent) were obtained with Sigma-Aldrich.

### Determination of phytochemical groups of aqueous extract, ethyl acetate and butanol fraction of *Acacia nilotica* var *adansonii*

#### Total phenolics quantification

The determination of total phenolics carried out according to the technique of Singleton et al<sup>7</sup>. In a test tube containing 1 ml of 1 mg/ml concentration extract, 1 ml of 2N FCR (Folin Ciocalteu reagent) and 3 ml of a 20% sodium carbonate solution added. The mixture made in triplicate with also a white control with distilled water. After 40 min incubation at room temperature of the mixture, the absorbance at 760 nm measured with the spectrophotometer. From the standard curve plotted with tannic acid, the total phenol concentration of the extract provided by the formula:

$$T_{PT} = (C_{tube} / C_i) \times D$$

$T_{PT}$ : total phenol content of the extract expressed in tannic acid equivalent (EAT)/g;  $C_{tube}$ : is the concentration in mg EAT/mL in the assay tube;  $D$ : the dilution factor;  $C_i$ : the concentration in mg/mL in the stock solution

#### Quantification of tannins

The method described by Tibiri et al<sup>8</sup> adopted for the determination of tannins. It based on the precipitation of tannins with polyvinyl polypyrrolidone (PVPP) by the formation of a complex. The complex of 2 mg of tannin requires 100 mg of PVPP. Thus, in a 1ml volume of an extract of concentration 1mg / ml, a sufficient amount of PVPP added there to and then stirred. After 15 minutes incubation at 4 °C., the mixture centrifuged at 3000 g for 10 minutes. As the tannins precipitated, the supernatant was recovered for a total phenolics assay other than tannins<sup>7</sup>. The difference between the first value of the total phenolic compounds (which contained the tannins) and the second value of the total phenolics (in the absence of the tannins) gives the tannin content.

#### Quantification of flavonoids

The total flavonoids was determined according to the method described by Abdel-Hameed<sup>9</sup>. In a test tube, a

mixture of 100 µl of an extract of concentration 1 mg/ml in water was prepared with 100 µl of 2% aluminium trichloride (AlCl<sub>3</sub>) in methanol (CH<sub>3</sub>OH). The volume of the mixture brought to 5 mL with methanol after adding a drop of acetic acid (CH<sub>3</sub>COOH). A white witness made parallel with the water. The mixtures allowed incubating for 40 min before measuring the absorbance at 415 nm spectrophotometer. A standard curve plotted with quercetin and used as the reference compound. The content of flavonoids in the extract is determined in Equivalent Quercetin (EQ) according to the following formula:

$$T_{Flav} = (C_{tube} / C_i) \times D$$

$T_{Flav}$ : the total flavonoid content of the extract expressed in equivalent quercetin (EQ)/g;  $C_{tube}$ : the concentration in mg EQ/mL in the assay tube;  $D$ : the dilution factor;  $C_i$ : the concentration in mg/mL in the stock solution

### Antioxidant activity

#### Evaluation of the anti-radical activity by radical DPPH° inhibition (2,2-Diphénylpicrylhydrazine)

It performed according to the method described by Kim et al<sup>10</sup>. A cascade dilution of the extracts and Trolox (positive control) of concentration 1 mg/mL were performed. A solution of DPPH (4mg in 100mL of methanol) was prepared. Then, on a 96-well microplate, a reaction mixture of 20 µL of each dilution of the extracts and Trolox with 200 µL of the DPPH solution made. The mixture allowed incubating for 30 minutes with methanol used as white. The absorbance reading is then 490 nm to the photo spectrometer (Agilent 8453). The percentage inhibition calculated according to the formula:

$$\% \text{Inhibition} = (A_0 - A_1/A_0) \times 100$$

$A_0$ : the absorbance of the negative control;  $A_1$ : the absorbance of the sample

The antiradical power (ARP) was calculated by the formula:

$$ARP = 1/IC_{50}$$

**ARP**: anti-radical power; **IC<sub>50</sub>**: inhibitory concentration of 50% DPPH° expressed in µg/mL of extract.

#### Evaluation of the anti-radical activity by the radical ABTS reduction

In an Erlenmeyer flask containing 5 ml of distilled water, 12.2 mg of ABTS [2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)] are dissolved therein. Then 3,312mg of potassium persulfate added. The mixtures were obtained kept for 12 to 16 hours of time away from light and at room temperature. A volume of 4.5 ml of the mixture diluted in 220 mL of ethanol for carrying out the ABTS test. According to the method described by Re et al<sup>11</sup>, a cascade dilution of the extracts and Trolox (positive control) of concentration 1 mg/mL were made. On a 96-well microplate, a reaction mixture is prepared with 20 µL of each dilution and 200 µL of the ABTS solution plus diluted in triplicate. The reaction mixture was allowed incubating at room temperature for 30 minutes, in the dark. Activity monitoring did at the photo spectrometer at 432nm wavelength. The following formula used to determine the inhibition percent:

$$\% \text{Inhibition} = (A_0 - A_1/A_0) \times 100$$

$A_0$ : is the absorbance of the negative control;  $A_1$ : the absorbance of the sample.

The anti-radical power (ARP):

$$ARP = 1/IC_{50}$$

### Evaluation of the reducing power of iron (FRAP)

The method described by Apati et al<sup>12</sup> which were adopted. In a volume of 0.5 mL of extract and concentration 1 mg / mL, a volume of 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium hexacyanoferrate [K<sub>3</sub> Fe(CN)<sub>6</sub>] 1% in water added. After incubating the mixture in a water bath at 50° C. for 30 minutes, 1.25 mL of trichloroacetic acid (10%) added thereto. Then the whole was centrifuged at 3000 rpm for 10 minutes. After the centrifugation, a volume of 0.625 mL of the mixture diluted in 0.625 mL of distilled water contained in an Eppendorf tube. 0.125 mL of freshly prepared 1% FeCl<sub>3</sub> in water added for instantaneous reading at the 700 nm photometer. From a standard curve of established ascorbic acid, the reducing power of the extract was determined and expressed in mmol Equivalent ascorbic acid (EAA) per gram of dry extract (mmol ascorbic acid / g dry extract) according to the formula:

$$C = (c \times D/M \times Ci) \times 1000$$

**C**: concentration of reducing compounds in mmol EAA/g of solids ; **c**:concentration of the extract read ; **D** : dilution factor of the stock extract solution ; **Ci** : concentration of the mother extract solution ; **M** : molar mass of ascorbic acid (176.12g/mol).

### Antispasmodic activity

The use of the rat for the test was in accordance with internationally accepted principles for the use and care of laboratory animals as defined by the European Community Directives (1986 EEC Directive, 86/609 / EEC). This study carried out according to the method described by Magnus<sup>5</sup>. It consists in evaluating the relaxing effect of the extract, on a contraction phase of contraction of the isolated rat

duodenum, induced by a contracting agent. To evaluate the relaxing effect of the aqueous extract and fractions, cumulative concentrations of each extract (260 µg/mL, 560 µg/mL, 1160 µg/mL, 1960 µg/mL, 2960 µg/mL) injected into the isolated organ the isotonic phase and then in contraction tonic phase of the duodenum induced with acetylcholine (1 mM) or BaCl<sub>2</sub> (160 µg / mL). The peak obtained at the recorder level with each extract compared with that previously created by acetylcholine (Ach). The references used were atropine and papaverine. The percentage inhibition of contraction (PI) calculated according to the formula:

$$PI = (h_1 - h_2 / h_1) \times 100$$

**h<sub>1</sub>** : height of the peaks due to the contractor alone; ; **h<sub>2</sub>** : heights of the peaks due to the contractor in the presence of the extract.

### Analysis of the Results

Calculations of percentages of inhibitions and equivalents of tannic acid, quercetin and ascorbic acid performed with the MS Excel software (CORREL statistical function). The results of the pharmacological study expressed as mean ± Standard Mean Error (E.S.M.). The different figures were plotted using GraphPad Software Prism version 5.01. The series considered significant when the probability of error (p) is lower than the agreed risk: 0.05 (p <0.05).

## RESULTS

### Quantification of total polyphenols, tannins and total flavonoids

Total phenolic, tannin and flavonoid content of extract and fractions showed in Table 4.

Table 2: Total phenolic, tannin and flavonoid content

	Phenols T (mg ETA/g)	Tannin (mg ETA/g)	Flavonoid (mg QE/g)
AE	573.03±9.88	519.98±8.86	13.65±8.08
EAF	948.94±5.05	633.37±1.96	10.32±2.39
BF	769.23±21.96	625.23±21.75	20.71±1.29

EAF: ethyl acetate fraction; BF: butanolic fraction; AE: Aqueous extract; ETA: Equivalent tannic acid; EQ: Quercetin Equivalent

### Antioxidant assay

#### Evaluation of the anti-radical activity DPPH°

The better anti-radical activity obtain with ethyl acetate fraction It's better than de Trolox. Aqueous extract showed a low anti-radical activity.

#### Evaluation of the anti-radical activity ABTS

ABTS test showed good anti-radical activity of the ethyl acetate fraction and Aqueous extract. However, their anti-radical activity was less than Trolox anti-radical activity

#### Reducing power by the FRAP method (Ferric Reducing Antioxydant Power)

The reducing power of the iron extracts was expressed in mole equivalent ascorbic acid. The ethyl acetate fraction expressed the highest reducing, followed by the butanol fraction and the aqueous extract.

Table 4: Summary of antioxidant activity values DPPH, ABTS, FRAP

Extract	DPPH		ABTS		FRAP
	IC <sub>50</sub> (µg/mL)	ARP	IC <sub>50</sub> (µg/mL)	ARP	mmol EAA/mL
AE	8.19±0.87	0.12	5.81± 0.11	0.17	1231.46±0.23
EAF	2.07± 0.19	0.48	5.65± 0.02	0.18	1421.81±0.07
BF	4.33±0.08	0.23	10.86±0.17	0.09	1390.87±0.02
Trolox	5.69±0.21	0.18	3.78±0.06	0.27	

EAF: ethyl acetate fraction; BF: butanolic fraction; AE: Aqueous extract; EAA: Equivalent ascorbic acid; ARP: Anti-radical Power

**In vitro study of antispasmodic activity of aqueous extract, ethyl acetate and butanol fraction of *Acacia nilotica* var *adansonii*, on intestinal smooth muscle**

**Effect of extracts and atropine on acetylcholine-induced duodenum contraction**

Different concentrations of the aqueous extract and the butanolic fraction induced the relaxation of the muscle. Aqueous extract showed good relaxation ( $IC_{50} = 13.02 \mu\text{g/mL}$ ; Fig 1) than butanolic fraction ( $IC_{50} = 96.03 \mu\text{g/mL}$ ; Fig 2). It is however, less better than atropine relaxation induced ( $IC_{50} = 1.018 \mu\text{g/mL}$ ; Fig 3). The ethyl acetate fraction did not show effect on muscle contraction.

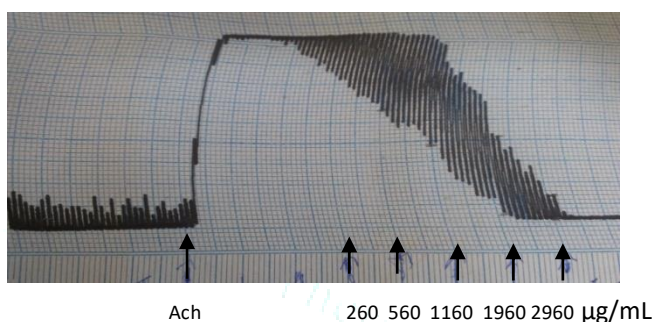
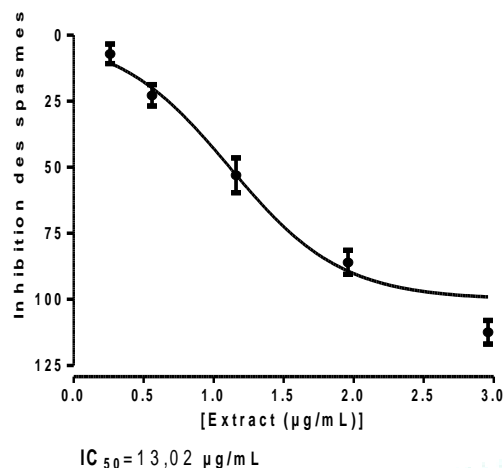


Figure 1 : Dose-effect profile of aqueous extract relaxation on Ach-induced

Photo 1 : Inhibition of ach-induced smooth muscle contraction by extract.

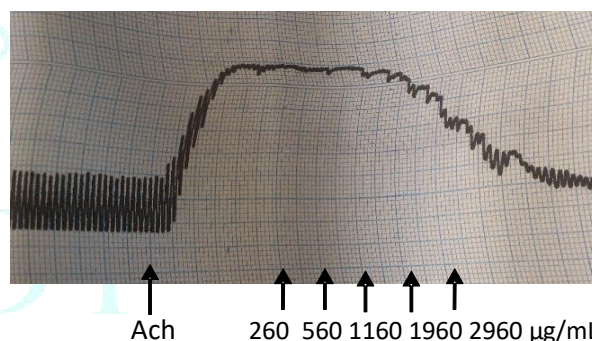
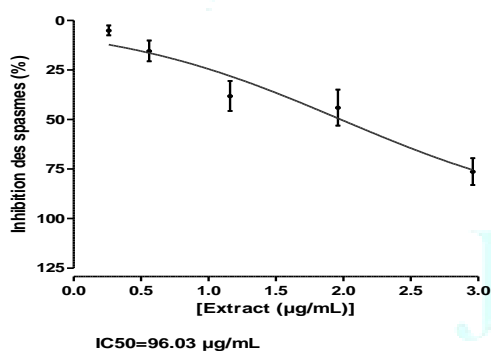


Figure 2 : Dose-effect profile of butanol fraction relaxation on Ach-induced

Photo 2: Inhibition of ach-induced contraction by butanol fraction

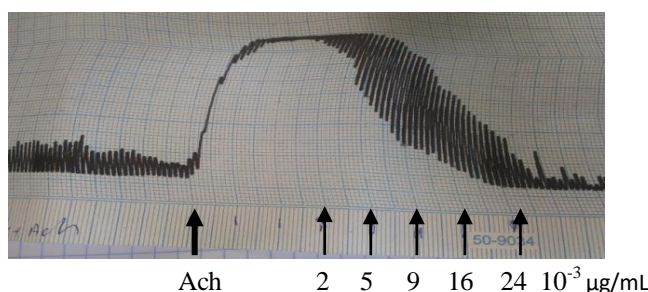
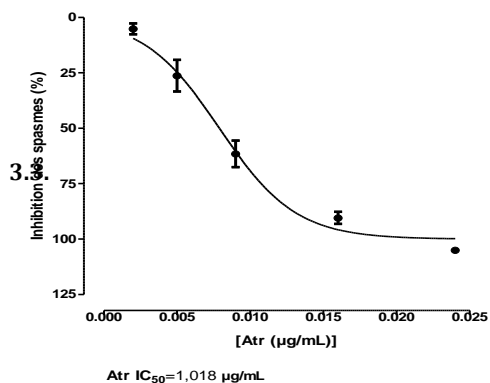


Figure 3: Dose-effect profile of atropine relaxation on Ach-induced

Photo 3: Inhibition of ach-induced contraction by atropine



### Effect of extracts and papaverine on BaCl<sub>2</sub>-induced duodenum contraction

Only aqueous extract (Fig 4) has been get inhibitory effect on the duodenum contraction, induced by 160 µg/mL BaCl<sub>2</sub> (IC<sub>50</sub> = 117.2 µg/mL). The ethyl acetate and butanol fraction did not show any effects with BaCl<sub>2</sub> contraction (Data not show). Nevertheless, papaverine showed good relaxation (IC<sub>50</sub> = 2.054 µg/mL) with BaCl<sub>2</sub> induction (Fig 5).

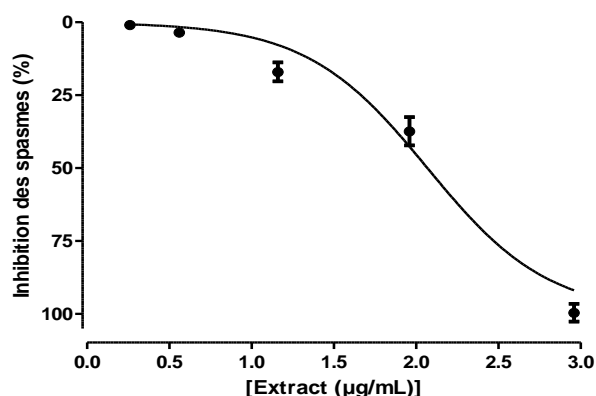


Figure 4: profile of atropine relaxation on BaCl<sub>2</sub> - induced by the extract

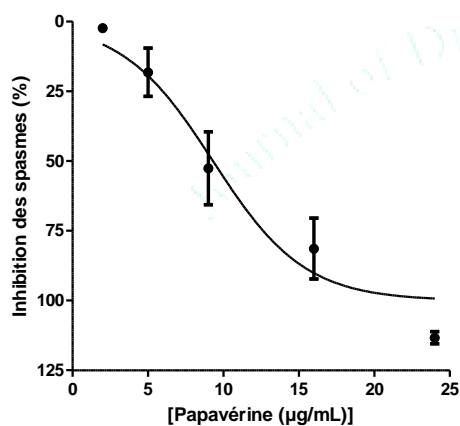


Figure 5: profile of atropine relaxation on BaCl<sub>2</sub> - induced by the papaverine

The phytochemical groups quantification showed that, aqueous extract have a higher tannins content (519.98 ± 8.86 mg EAT/gE) than flavonoids (13.65 ± 8.08 mg EQ / g). This means that more than half of the polyphenols measured would be tannins. Flavonoids would represent less than 10%. It is also remark by Mukundi et al, in the hydroethanolic leaf extract where the total phenolic content was 2.16 mg / g with 2.06 mg / g tannin content and 0.29 mg / g dry matter total flavonoids<sup>13</sup>. According to Serémé et al., the bark of *Acacia nilotica* trunks is rich in tannins after these fresh fruits among the tanniferous plants of Burkina Faso, ie 28.7% of the weight of the dry matter<sup>14</sup>. Conformity therefore, the name of "tannin plant". In the methanolic extract of the *Acacia nilotica* var *nilotica* trunk bark<sup>15</sup>, the total phenolic content (945mg EAG / g) is more than aqueous extract of the *adansonii* variety (519.98 ± 8, 86 mg EAT / g). Omara et al<sup>16</sup>, also remarked this high total phenolic content in fruit of *Acacia nilotica* (504.99 mg EAG / g) with a flavonoid content equal to 243 mg Rutin equivalent / g.

The low proportion of flavonoids in the polyphenols found is different to the results obtained by Sadiq et al., with hydroethanolic extract of the bark of the *nilotica* variety<sup>17</sup>. It was 62.03 mg EAG / g polyphenol content and 45.5mg EQ / g

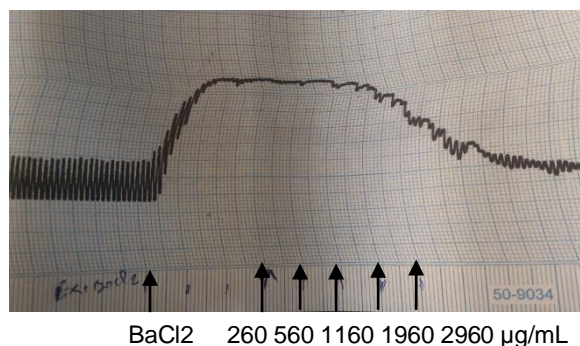


Photo 4: Inhibition of BaCl<sub>2</sub>-induced contraction by the extract

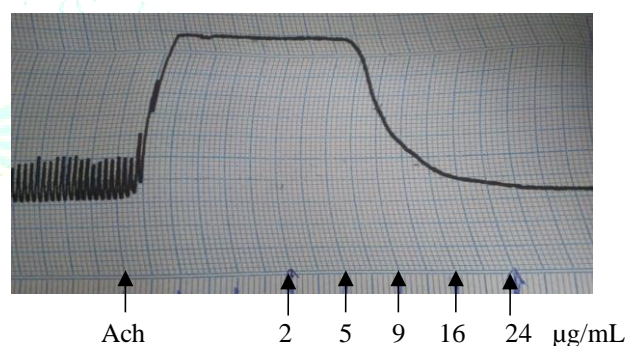


Photo 4: Inhibition of BaCl<sub>2</sub>-induced contraction by the papaverine

for flavonoids. Flavonoids content represented more than half of the polyphenols in this study. It could be explain by the difference in the plant variety. The *nilotica* variety bark, therefore, has a higher flavonoid content than *adansonii* variety.

Antioxidant assay by antiradical methods (DPPH and ABTS) and iron reduction method (FRAP), allows better evaluation of the antioxidant potential of the extracts. The ethyl acetate fraction showed both of DPPH (IC<sub>50</sub> = 2.07 ± 0.19 µg / mL) and ABTS (IC<sub>50</sub> = 5.65 ± 0.02 µg / mL), better antiradical activity than butanolic fraction and aqueous extract. To the DPPH free radical test, the fraction of ethyl acetate and the butanolic fraction (IC<sub>50</sub> = 4.33 ± 0.08 µg / mL) presented better antiradical activity than Trolox (IC<sub>50</sub> = 5.69 ± 0.21 µg / mL). However, in the ABTS test, the antiradical activity of Trolox (IC<sub>50</sub> = 3.78 ± 0.06 µg / mL) were better than of ethyl acetate fraction (IC<sub>50</sub> = 5.65 ± 0.02 µg / mL) and the butanol fraction (IC<sub>50</sub> = 10.86 ± 0.17µg / mL). The aqueous extract had a good antiradical activity on ABTS radical (IC<sub>50</sub> = 5.81 ± 0.11 µg / ml) than on DPPH radical (IC<sub>50</sub> = 8.19 ± 0.87 µg / mL). This means that, fractions and aqueous extract have hydrogen proton donor chemical groups<sup>18</sup>. The antiradical activity of the aqueous extract of the *adansonii* variety on the DPPH radical would be better than of methanolic extract

of the leaves ( $IC_{50} = 350 \mu\text{g} / \text{mL}$ ) and barks ( $IC_{50} = 1410 \mu\text{g} / \text{mL}$ ) of the nilotica variety<sup>17</sup>. Furthermore the ABTS antiradical activity of the butanolic fraction ( $IC_{50} = 12.74 \pm 0.01 \mu\text{g} / \text{mL}$ ) and the ethyl acetate fraction ( $IC_{50} = 12.73 \pm 0.01 \mu\text{g} / \text{mL}$ ) are lower than of the bark extract of the adansonii variety<sup>19</sup>. To the DPPH radical, antiradical activity of the extracts of the bark of the adansonii variety is better than of butanolic fraction ( $IC_{50} = 83.3 \pm 1.15 \mu\text{g} / \text{mL}$ ), ethyl acetate ( $IC_{50} = 90.26 \pm 2.41 \mu\text{g} / \text{mL}$ ) and the aqueous fraction ( $IC_{50} = 59.15 \pm 0.87 \mu\text{g} / \text{mL}$ ) of the nilotica variety<sup>19</sup>. The iron reduction test (FRAP) showed high reducing power of the ethyl acetate fraction ( $1421.81 \pm 0.07 \text{ mmol EAA} / \text{mL}$ ), butanolic fraction ( $1390.87 \pm 0.02 \text{ mmol EAA} / \text{mL}$ ) and aqueous extract ( $1231.46 \pm 0.23 \text{ mole EAA} / \text{mL}$ ). These activities are higher than of gallic acid ( $18.46a \pm 1.51 \text{ mmol EAA} / \text{mL}$ ) and quercetin ( $13.19 \pm 2.17 \text{ mmol EAA} / \text{mL}$ ) activities<sup>20</sup>. This reducing power of acacia nilotica extract, linked to the presence of reductone molecules<sup>21</sup>. These reductones would act on the free radical chains by donation of hydrogen atom causing their breaks<sup>22</sup>. In all three antioxidant activities, aqueous extract and fractions had good antioxidant activity. The phenolic compounds and an especially high gallic acid content would be responsible, for this strong antioxidant activity of bark extract aqueous of *Acacia nilotica* var *adansonii*. The results indicates a close link between the antioxidant activity and total phenol content of the extracts. This correlation between antioxidant activity and total phenolic content evoked and proven by many authors<sup>23,24,25</sup>.

Aqueous extract of *Acacia nilotica* var *adansonii* and butanolic fraction inhibit acetylcholine-induced contraction on smooth muscle of rat duodenum. This interaction with cholinergic system means that aqueous extract and the butanolic fraction are acetylcholine blockers. The effective inhibitory concentration ( $IC_{50}$ ) of the aqueous extract was  $13.02 \mu\text{g} / \text{mL}$  and of butanol fraction was  $96.03 \mu\text{g} / \text{mL}$ . Atropine showed an  $IC_{50} = 1.02 \mu\text{g} / \text{mL}$ . This means that aqueous extract is just 10 times less potent than atropine. The compounds of the aqueous extract could also act as atropine. They could block the Ach by direct competition on muscarinic M3 receptors. Further investigation required. Because it could be another indirect anticholinergic pathway action. Such as inhibition of calcium mobilization in calcium channels. The butanol fraction is less potent than the aqueous extract and almost 10 times less than atropine. Furthermore, its relaxation effect does not reach 100%, unlike aqueous extract and atropine. It would mean that fractionation causes the decrease of the anticholinergic activity of the aqueous extract. This confirmed by the absence of anticholinergic effect with ethyl acetate fraction. The aqueous extract is also shown to be a better inhibitor of Ach compared to certain plants, such as *Erythrina indica* lam ( $IC_{50} = 79.7 \mu\text{g} / \text{mL}$ )<sup>26</sup>, *Piper sarmentosum* ( $IC_{50} = 88 \mu\text{g} / \text{mL}$ )<sup>27</sup>, *Blumea lacera* ( $IC_{50} = 76.19 \mu\text{g} / \text{mL}$ ).

In addition, aqueous extract of *Acacia nilotica* inhibited smooth muscle contraction, by  $\text{BaCl}_2$ -induced. The inhibitory concentration  $IC_{50} = 117.2 \mu\text{g} / \text{mL}$ . This concentration of inhibition is significantly higher than of papaverine which was  $IC_{50} = 2.054 \mu\text{g} / \text{mL}$ . It means that inhibitory effect of aqueous extract less potent than of papaverine. The aqueous extract would also act on myocytes to allow smooth muscle relaxation. It could act as papaverine, inhibiting the functioning of phosphodiesterase. This way of action on myocytes said musculotropic. It is better than plant *Blumea lacera* ( $IC_{50} = 120.2 \mu\text{g} / \text{mL}$ )<sup>28</sup> or *Teucrium stocksianum* which have no effect by this musculotropic pathway<sup>29</sup>. According to Zhu et al<sup>30</sup>, tannins could be responsible for the spasmolytic activity of the aqueous extract and flavonoids<sup>31</sup>.

However, this spasmolytic effect of flavonoids and tannins does not appear proportionally to their contents in the extracts. Because only the aqueous extract whose tannins and flavonoids content were low compared to ethyl acetate and butanol fractions, showed a more significant spasmolytic effect. This means that the spasmolytic effect of the aqueous extract could result from other phytochemical groups such as glycosides<sup>31</sup> or alkaloids. The effect could also result from a synergy action between the different phytochemical groups, which make up the extract.

## CONCLUSION

The results of this study indicate that the aqueous extract of the trunk bark powder of *Acacia nilotica* var *adansonii* has both antispasmodic and antioxidant properties compared to its ethyl acetate fraction and butanol. The measured and identified polyphenol groups or other phytochemical groups would be responsible for these antioxidant and antispasmodic properties of the aqueous extract. Therefore, it a plant, which used in traditional medicine against parasitosis and diarrhoea justified. Further investigations, will be better to demonstrate the antispasmodic and antiparasitic action mechanism of the aqueous extract of *acacia nilotica* var *adansonii*.

**CONFLICTS OF INTEREST:** none

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## REFERENCES

1. Nacoulma O., Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso : cas du plateau central. Thèse de Doctorat ès Sciences Naturelles, Faculté des Sciences et Techniques de l'Université de Ouagadougou, 1996 ; 320 p.
2. Traore A., Ouedraogo Sylvain, Lompo M., Traore S., Some N., Guissou I.P., Ethnobotanical survey of medicinal plants used to treat gastrointestinal parasites in human and livestock in four geographic areas of Burkina Faso (West Africa), Scholars Research Library, 2013; 5 (6):172-177.
3. Boly A.G.L., Belemlilga M., Traoré A., Ouédraogo S., Guissou I.P., Phytochemical and in vitro anthelmintic properties studies of *Acacia nilotica* var *adansonii* (Guill & Perr). O Ktze (MIMOSACEAE) trunk barks aqueous extract, International journal of pharmacognosy and phytotherapy research, 2018;10 (1): 5-10
4. Saini M.L, Comparative Pharmacognostical and antimicrobial studies of *Acacia* species (Mimosaceae). Journal of Medicinal Plants Research., 2008; 2(12):378-386.
5. Magnus In., Pharmacological experiments on isolated preparations Churchill Livingstone. « Staff of the department of pharmacology », University of Edinburgh, 1970; 62p.
6. Guinko S, Etude sur le rôle des *Acacia* dans le développement rural au Burkina Faso et au Niger, IRD Editions Ouagadougou,1991; 36p.
7. Singleton V. L., Orthofer R., Lamuela-Raventos R. M, Analysis of phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent, Methods in Enzymology, 1999; 299: 152-178.
8. Tibiri. A., Rakotonandrasana. O., NACOULMA. G. O., BANZOUZI. J. T, Radical Scavenging Activity, Phenolic Content and Cytotoxicity of *Entada africana* Guill. Et Perr. (Mimosaceae), Journal of Biological Sciences, 2007; 7(6): 959-963
9. Abdel Hameed, El-Sayed Saleh, Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chemistry, 2008; 114(4):1271-1277.
10. Kim, K.S., Lee, S., Lee, Y.S., Yung, S.H, Park, Y., Shin, Kh., Kim, B-K, Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*, J.Ethnopharmacol., 2003; 85: 69-72.
11. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C, Antioxidant activity applying an improved ABTS

- radical cation decolorization assay, Free Radical Biology and Medicine, 1999; 26(9-10):1231-1237.
12. Apati P., Szentmihalyi K., Kristo S. T., Papp I., Vinkler P., Szoke E., Kery A, Herbal remedies of Solidago correlation of phytochemical characteristics and antioxidative properties, Journal of Pharmaceutical and Biomedical Analysis, 2003; 32(4-5): 1045-1053
  13. Mukundi MJ, Piero NM, Mwaniki NEN, Murugi NJ, Daniel AS., Antidiabetic Effects of Aqueous Leaf Extracts of *Acacia nilotica* in Alloxan Induced Diabetic Mice, J. Diabetes Metab., 2015; 6: 568.
  14. Serémé A., Millogo J., Guinko S., Nacro M, Concentration à tanins des organes de plantes tannifères du Burkina Faso, J. Soc. Ouest-afric. Chim, 2008; 025: 55-61.
  15. B Singh., Sukhpreet Singh., Neeraj Kumar., Subodh Kumar., Saroj Arora, Umbelliferone – An antioxidant isolated from *Acacia nilotica* (L.) Willd. Ex. Del. Rajbir, Food Chemistry, 2010; 120 : 825–830.
  16. Omara A., Somaia A., Nada Abdel Razik., Farrag H., Walid M, Therapeutic effect of *Acacia nilotica* pods extract on streptozotocin induced diabetic nephropathy in rat, Phytomedicine, 2012; 19: 1059- 1067.
  17. Sadiq M., Hanpithakpong W., Tarningb J., Kumar A Anal, Screening of phytochemicals and in vitro evaluation of antibacterial and antioxidant activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. Industrial Crops and Products, 2015; 77: 873–882.
  18. Patil A. P., Patil V. V., et Patil V. R. 2009, In-vitro free radicals scavenging activity of *Madhuca indica* Gme, Pharmacologyonline, 2009; 2:1344-1352
  19. Barapatre A., Singh A. M., Mekala S., Das A, Harit Jha, In vitro evaluation of antioxidant and cytotoxic activities of lignin fractions extracted from *Acacia nilotica*, International Journal of Biological Macromolecules. 2016; 01:109-115
  20. Bangou M J, Etude phytochimique et activités biologiques des tiges feuillées de *Lantana camara* L. et de *Lippia chevalieri* Moldenke: deux VERBENACEAE du BURKINA FASO, Thèse unique en Biochimie et Chimie des Substances Naturelles Université de Ouagadougou (Burkina Faso), 2012 ; 199p
  21. Duh PD., Tu YY, Yen GC, Antioxidant activity of the aqueous extract of *harn jwur* (*Chrysanthemum morifolium* Ramat). Lebensmittel-Wissenschaft and Technologie 1999; 32: 269-277.
  22. Gordon MH, The mechanism of antioxidant action in vitro. In: BJF Hudson (Ed.), Food antioxidants Elsevier Applied Science, London, 1990; 1–18.
  23. Surveswaran S., Cai Y-Z., Corke H., Sun M, Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry, 2007 ;102: 938-953
  24. Meda, N-T.R. Etude phytochimique et activités biologiques des galles et des feuilles de *Balanites aegyptiaca* (L.) Del. (Balanitaceae) utilisées en médecine traditionnelle au Burkina Faso. Thèse Unique, Université de Ouagadougou, 2010 ; 183p.
  25. Awah F.M., Uzoegwu P.N., Oyugi J.O., Rutherford J., Ifeonu P, Eze M.O, Free radical scavenging activity and immunomodulatory effect of *Stachytarpheta angustifolia* leaf extract. Food Chemistry, 2010; 119: 1409 – 1416
  26. Kamalraj R and Devdass, Antispasmodic studies on leaf extract of *Erythrina indica* Lam. International Journal of Research in Ayurveda & Pharmacy, 2011; 2(4): 1380-1382
  27. Thaina P., Poonpanang P., Kitja Sawangjaroen, Comparison of Spasmolytic Activities of *Piper longum*, *P. sarmentosum* and *Quercus infectoria* Extracts with Loperamide and Verapamil in Rat and Guinea Pig Intestinal Tissues, In : III WOCMAP Congress on Medicinal and Aromatic Plants, Traditional Medicine and Nutraceuticals 680, 2003; 6:183-189.
  28. Dixit A., P Tiwari., D Parasher., S Bhargava (2014): Antispasmodic studies on leaf and root extract of *Blumea lacera*. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 6, Issue 1.
  29. Niaz A., Wadood S., Shah A, Antispasmodic activity of *Teucrium stocksianum* boiss, Pakistan journal of pharmaceutical sciences. 2014; 24 (2) : 171-174.
  30. Zhu F, Huang B, Hu CY, Jiang QY, Lu ZG, Lu M, Wang MH, Gong M, Qiao CP, Chen W, Huang PH, Effects of total flavonoids of *Hippophae rhamnoides* L. on intracellular free calcium in cultured vascular smooth muscle cells of spontaneously hypertensive rats and Wistar-Kyoto rats. Chin J Integr Med. 2005; 11 (4): 287-292.
  31. Ko TF., Weng YM., Lin SB., Chiou RY., Antimutagenicity of supercritical CO2 extracts of *Terminalia catappa* leaves and cytotoxicity of the extracts to human hepatoma cells. J Agric Food Chem, 2003; 51(12): 3564-3567.



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