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Phytochemical screening, antioxidant and anti-diabetic activities of *Leptadenia hastata*

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Abstract

***Leptadenia hastata (L. hastata)* is traditionally used in Burkina Faso to treat some diseases. Leaves extracts was assessed for antioxidant activities and α-glucosidase inhibition.**

TLC analysis of fractions revealed the presence of quercetin in the ethyl acetate fractions of *L. hastata* and rutin in the n-butanol fraction. The results of radical scavenging activity showed that DCM and AcOET fractions exhibited FRAP values of 159.932 and 76.144 µg ET/mg and DPPH values of 60.379 and 46.569 µg ET/mg respectively. Among these extracts, DCM expressed the strongest activity on the enzymatic reaction regarding to the lowest value of IC_{50} close to 0.431 mg/mL followed by AcOET with an IC_{50} = 1.195 mg/mL and BuOH with an IC_{50} = 1.623 mg/mL in comparison of the standard acarbose with an IC_{50} = 0.215 mg/mL ± 0.004 mg/mL. These results show that leaves extracts of *L. hastata* have antidiabetic potentiality.

Keywords:* *Leptadenia hastata; Antioxidant; Diabetes; α-glucosidase

Introduction

Type 2 diabetes is a chronic disease resulting from an abnormality in carbohydrate metabolism. Diabetes is the leading cause of death from cardiovascular disease, amputation and blindness [1]. According to the International Diabetes Federation, the world's population will have 629 million diabetics in 2045 including 438 million diabetics aged 20 to 64 [2]. In sub-Saharan Africa, the prevalence is

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between 0.2% and 12% and in Burkina Faso, in 2013, it was 4.9% in the range 25 to 64 years and 7.1% in 2022 [3-5]. The precariousness of the conditions of care for diabetics as well as the socio-economic indigence in developing countries, particularly in Burkina Faso, are all obstacles to a normal blood sugar level, which is essential for the prevention of complications in diabetics [6]. The population of Burkina Faso, essentially rural, does not benefit from satisfactory health coverage. This is due to the high cost of modern medicines, which are generally inaccessible to all social classes. Indeed, 80% of the population lives from agriculture and livestock, sectors that are still very unprofitable and underdeveloped. And according to a report by the Burkinabe Ministry of Health in 2015, 43.9% of the population of Burkina Faso, at national level lived below the poverty line [4]. This lead to the use of medicinal plants for primary health care needs. Indeed, medicinal plants contain secondary metabolites with various biological activities which could contribute to of the treatment of diabetic people. After a meal, postprandial hyperglycemia is most pronounced in patients with diabetes. Inhibiting glucose uptake in the intestines could reduce postprandial state [7]. α-glucosidase is a key enzyme for the metabolism of oligosaccharides to absorbable monosaccharides in the small intestine [7]. So, inhibition of this enzyme may delay the digestion of carbohydrates, decreasing glucose uptake and therefore reducing postprandial hyperglycemia. The polyphenols are able not only to reduce oxidative stress but also to inhibit carbohydrate hydrolyzing enzymes and thus preventing hyperglycemia [8]. *L. hastata* contains secondary metabolites such as phenolics, flavonoids, tannins, alkaloids [9,10]. Phenolic compound contribute to antidiabetic activity and could inhibit α-glucosidase which is responsible for the hydrolysis of carbohydrates and therefore would reduce postprandial hyperglycemia [11,12]. Although the chemical composition has been documented, a study on the antidiabetic properties of *L. hastata* from Burkina Faso has not been done yet. Hence the aim of the present study that was to investigate in vitro the potential diabetic inhibitory properties of the leaves of *L. hastata* on α-glucosidase.

Materials and Methods

Plant material

The whole plant is known as a wild plant and recorded in the flora of Burkina Faso. In Burkina Faso legislation and in the interest of carrying out scientific studies to promote local plants, no authorization is required for scientific research activities. The plant only needs to be identified by botanists, registered and stored at the university herbarium.

For that, the leaves of *L. hastata* were collected in Ouagadougou at GPS coordinates 12°18'39.83"N; 1°30'1.97"W. The collected specimen of the leaves of *L. hastata* were identified by Amadé Ouedraogo, Full Professor of Botanic at the Laboratory of Biology and Vegetable Ecology of the University Joseph KI-ZERBO and were deposited in the herbarium of the University under the identification number 18014.

Dried plant material was pulverized using an electric grinder. The extraction was done using a protocol previously described by Karanga et *al.* (2017) with slight modifications [13]. Successive extraction has been made with increasing polarity from Dichloromethane (DCM), ethyl acetate (AcOET), n-butanol (BuOH). The different fractions and the residual aqueous (H_2O) fraction of *L. hastata* were obtained for further analysis.

Phytochemical screening

Flavonoids, quinones and tannins have been identified using classical methods like aluminum chloride, iron (III) chloride and soda test respectively. TLC was also realized.

Determination of total phenolic compounds

The content of total phenolic compounds present in a crude extract was determined by the Folin-Ciocalteu method, the principle of

which is the oxidation of the oxidizable groups of phenolic compounds by the Folin-Ciocalteu reagent [14]. The procedure consists in preparing solutions of different concentrations of the crude extract. To 60 µL of each of the above solutions is added 60 µL of RFC. After 8 min, 120 µL of 7.5% (w/v) Na_2CO_3 is added. The reaction mixture is incubated for 1 h at room temperature. Absorbances are read at 760 nm using a spectrophotometer (SPECTROstar NANO, BMG LABTECH).

Results are obtained by plotting absorbances against a calibration curve previously established using gallic acid as a reference and expressed in microgram equivalent of gallic acid per milligram extract (µg EAG/mg extract).

Determination of total flavonoids

This method involves complexing flavonoids with Al^{3+} ions from aluminum chloride ($AlCl_3$) in a basic medium. The method used is that of Chang et *al.* (2002) with slight modifications [15]. 50 µL of each sample, suitably diluted in MeOH, is mixed with 150 µL of distilled water, followed by 15 µL of 5% (w/v) $NaNO_2$. 5 min later, 15 µL of 10% (w/v) $AlCl_3$ is added. The whole mixture is incubated at room temperature for 6 min. 50 µL of 1N NaOH is added and the absorbance of the pinkish-colored mixture at 510 nm is measured using an absorbance meter. Spectrophotometer (SPECTROstar NANO, BMG LABTECH). A calibration curve was established using quercetin as a reference, following the same procedure as for the samples.

The flavonoid content of the sample, expressed as microgram quercetin equivalent per milligram extract (µg EQ/mg extract), is obtained by plotting the absorbance of the extracts against the quercetin calibration curve.

Determination of condensed tannins

This method involves the condensation of polyphenolic compounds with vanillin in an acid medium. Condensed tannins are determined for the various extracts according to the method of Price with a few modifications. To 400 µL of each sample or standard with appropriate dilutions, are added 3 mL of vanillin solution (4% in methanol) and 1.5 mL of concentrated HCl. After 15 minutes of reaction, absorbance is read at 500 nm using a spectrophotometer (SPECTRO star NANO, BMG LABTECH) [16].

Tannin concentrations are deduced from the calibration curve established with catechin (standard) and expressed in micrograms of catechin equivalent per milligram of extract (µg EC/mg extract).

FRAP method

FRAP assay was performed to investigate the reducing power. Piljac-Zegarac et *al.* (2009) method is used with slight modifications. At each 50 µL of the extract at different concentrations, 200 µL of FRAP reagent are added [17]. After 10 min incubation in laboratory temperature, the absorbance was measured of the intense blue stain at 595 nm using UV-vis spectrophotometer. This optical density is plotted against the calibration curve established using trolox. The results are expressed in micrograms equivalent of trolox per milligram of extract (µg ET/mg extract).

DPPH method

The radical scavenging activity was determined by using 2,2-diphenyl-1-picrylhydrazyl radical. Sanchez-Moreno (2002) method was used with slight modifications [18]. Thus, 50 µL of the extract is added to 200 µL of DPPH solution. After 10 min of incubation, the absorbance was measured at 515 nm with a spectrophotometer**.** Radical scavenging activity was expressed as IC_{50} in µg/mL.

Assay for α-glucosidase inhibitory activity

Inhibition of fractions on α-glucosidase activity evaluated using Ranilla et *al.* (2010) method with slight modifications [19]. The principle of this method is presented in **figure 1**. In fact, the antidiabetics present in the fractions will inhibit α-glucosidase enzyme. The more active the fractions, the lower the quantity of p-nitrophenol released a compound that we monitor in UV-Vis spectrometry at

405 nm.

Thus, in a mixture containing 20 µL of α-glucosidase (1 unit/mL) and 120 µL of 0.1 M phosphate buffer pH 6.9, 10 µL of each plant fraction at different concentrations is introduced. Each solution obtained is pre-incubated in 96-well microplates at 37°C for 15 minutes. After incubation, enzymatic reaction is initiated by adding 20 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) and the reaction mixture is incubated for a further 15 minutes at 37°C. The reaction is stopped by adding 80 µL of 0.2 M sodium carbonate solution. The absorbance is readed at 405 nm by a spectrophotometer of the type SPECTROstar NANO, BMG LABTECH, Ortenberg, Germany. The reaction system without plant extracts is used as a control and the system without α-glucosidase is used as a blank to correct the background absorbance. Acarbose is used as a positive control. The inhibition rate of α-glucosidase is calculated by the following formula:

$$\%\ of\ inhibition = \frac{Ac - As}{Ac} \times 100$$

Ac: Absorbance of the control

As: Absorbance of the sample

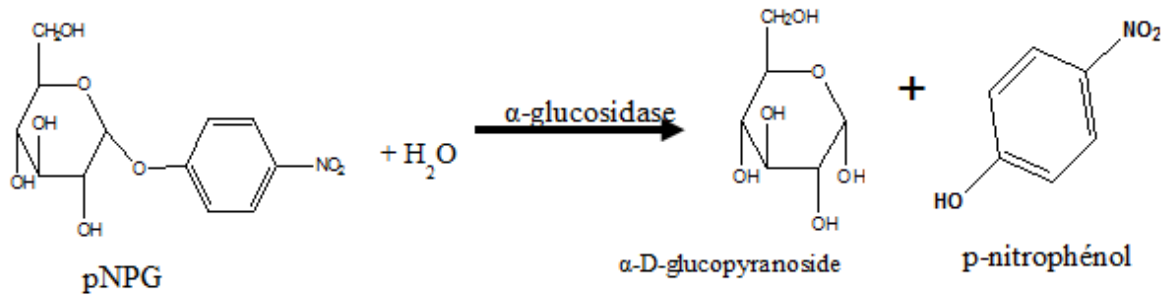

FIG. 1. **Principle of α-glucosidase inhibition.**

Results and Discussion

Chemical screening

The phytochemical screening revealed that the leaves extracts of *L. hastata* contain tannins, quinones and flavonoids (**TABLE 1**). Only the DCM (dichloromethane) fraction did not contain quinones.

TABLE 1: **Results of the phytochemical screening.**

Chemical groups	***L. hastata***			
	DCM	**AcOET**	**BuOH**	**H_2O**
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Quinones	-	+	+	+
(+) : positive test; (-) : négative test				

The Thin Layer Chromatography (TLC) performed confirmed the presence of flavonoids in the different fractions after spraying with Neu's reagent (**FIGURE 2**).

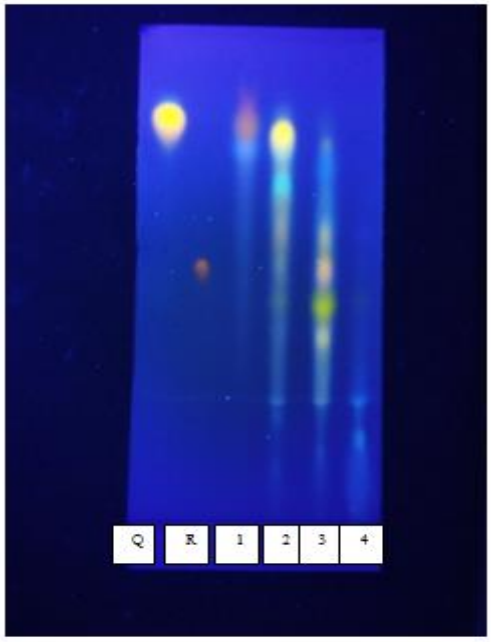

FIG. 2. **TLC of different fractions of *L. hastate.***

Q: quercetin standard, R: rutin standard; For L. hastata (1: DCM fraction, 2: AcOET fraction, 3: BuOH fraction, 4: residual H_2O fraction); mobile phase: Butanol-Acetic acid-water (4-1-5 v/v)

The frontal references were calculated and recorded in Table 2.

TABLE 2: **TLC of different fractions of *L. hastata*, *quercetin* and *rutin*.**

L. hastata		
Standard and Fractions	**Frontal References**	**Spot Light Colours**
Quercetin	0.81	Yellow
Rutin	0.51	Red
DCM	0.76	Blue
	0.81	Red
AcOET	0.19	Blue
	0.7	Turquoise
	0.81	Yellow
BuOH	0.14	Blue
	0.37	Beige Pink
	0.43	Greenish Yellow
	0.51	Red
	0.6	-
	0.69	Blue
	0.73	Blue
H_2O	0.69	Blue
	0.73	Blue

The analysis of **TABLE 2** indicates the presence of quercetin in the ethyl acetate fraction at the frontal reference of 0.81 and rutin in the butanol fraction of *L. hastata* at the frontal reference of 0.51.

Phenolic compound content

Total Phenolic Compound (TPC) content of each fraction was assessed and the results obtained are presented in the figure 3.

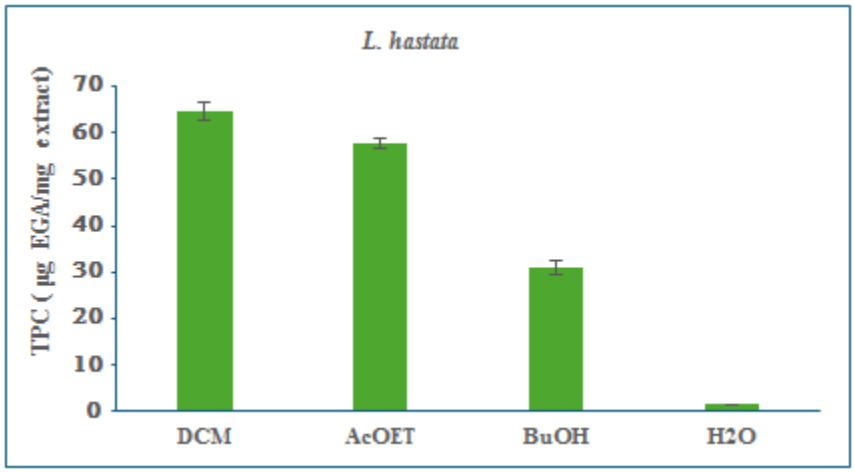

FIG. 3. **Histograms of total phenolic content.**

Figure 3 shows that DCM contains more phenolic compounds with 64.5 µg EGA/mg of extract, followed by ethyl acetate fraction 57.5 µg EGA/mg of extract, butanol fraction 30.9 µg EAG/mg of extract. The residual fraction H_2O contains the lowest amount which is close to 1.5 µg EGA/mg of extract.

Total flavonoid content

The evaluation of the Total Flavonoid Content (TFC) of the fractions of the plant is represented in the **Figure 4**.

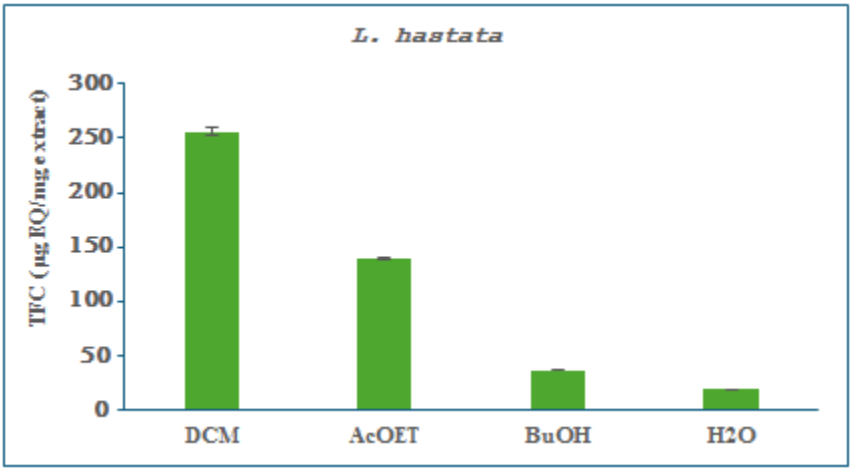

FIG. 4. **Histogram of total flavonoid content.**

As illustrated in the **Figure 4**, the DCM fraction contains 255.738 µg EQ/mg ± 4.01 µg EQ/mg of TFC, corresponding to the high value compared to the other fractions. This result is in agreement with the studies reported by Ganamé et *al.* (2020) [20].

Condensed tannin content

FIGURE 5 shows the results for Condensed Tannins Content (CTC).

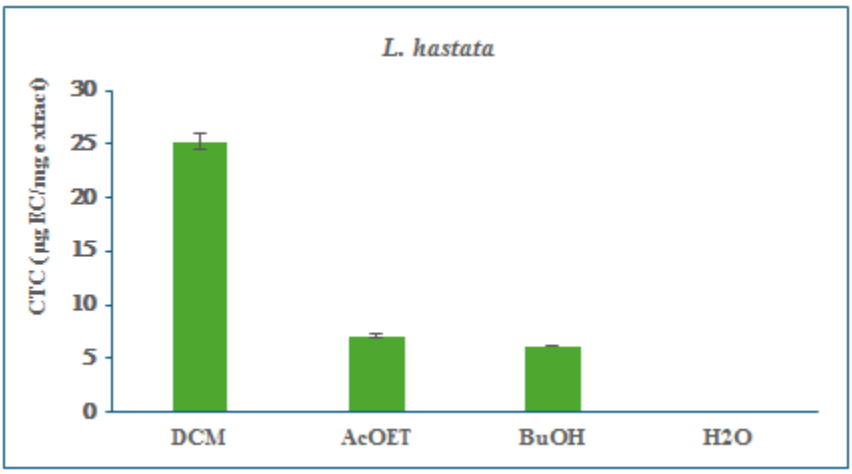

FIG. 5. **Histogram of condensed tannin content.**

It appears in this **FIGURE** that DCM fraction contains the highest content of condensed tannins which is 25.2 µg EC/mg of extract followed by 7.1 and 6.2 µg EC/mg of extract for AcOET and BuOH respectively.

Antioxidant activity

FIGURE 6 illustrates the results obtained from the evaluation of the antioxidant activities (FRAP and DPPH) of *L. hastata*.

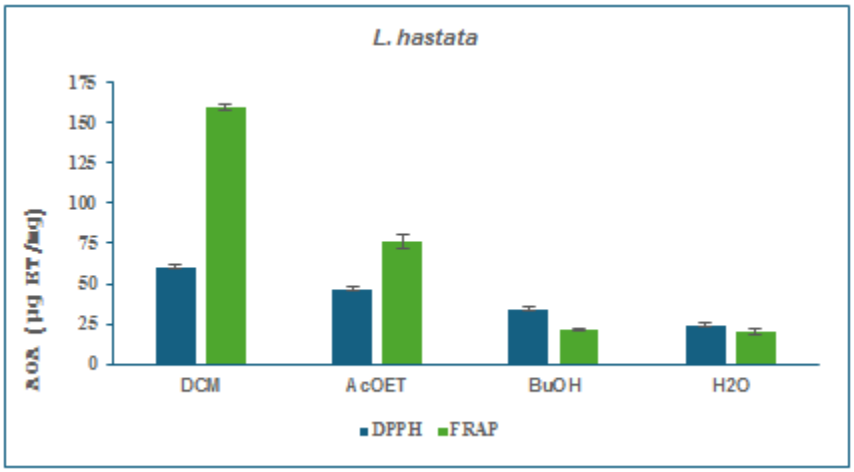

FIG. 6. **Antioxidants content using DPPH and FRAP methods.**

The results obtained show that DCM has the highest antioxidant content, followed by AcOET and BuOH using DPPH method. The contents are estimated at 60.379 µg ET/mg, 46.569 µg ET/mg and 34.262 µg ET/mg of extract respectively for DCM, AcOET and

BuOH. Using the FRAP method, the same tendance is observed. Indeed, the antioxidant contents are evaluated at 159.93, 76.14 and 21.24 for DCM, AcOET and BuOH respectively. These results agree with results obtained previously and could be explained by the high content of TPC and TFC. The high antioxidant activity is observed for DCM fraction of *L. hastata* which possesses high quantity of phenolic compounds and total flavonoids. These results are in agreement with the research work of Quezada and Zhang who reported similar results. It is well known that the use of antioxidants by diabetic's people could prevent long-term complications that may arise from diabetes [20-22]. So, the presence of antioxidant properties in these fractions could explain the use of *L. hastata* in the treatment of diabetes by population.

α-Glucosidase inhibitory activity

α-glucosidase inhibition has been evaluated on *L. hastata* fractions and the obtained results are shown on **FIGURE 7.**

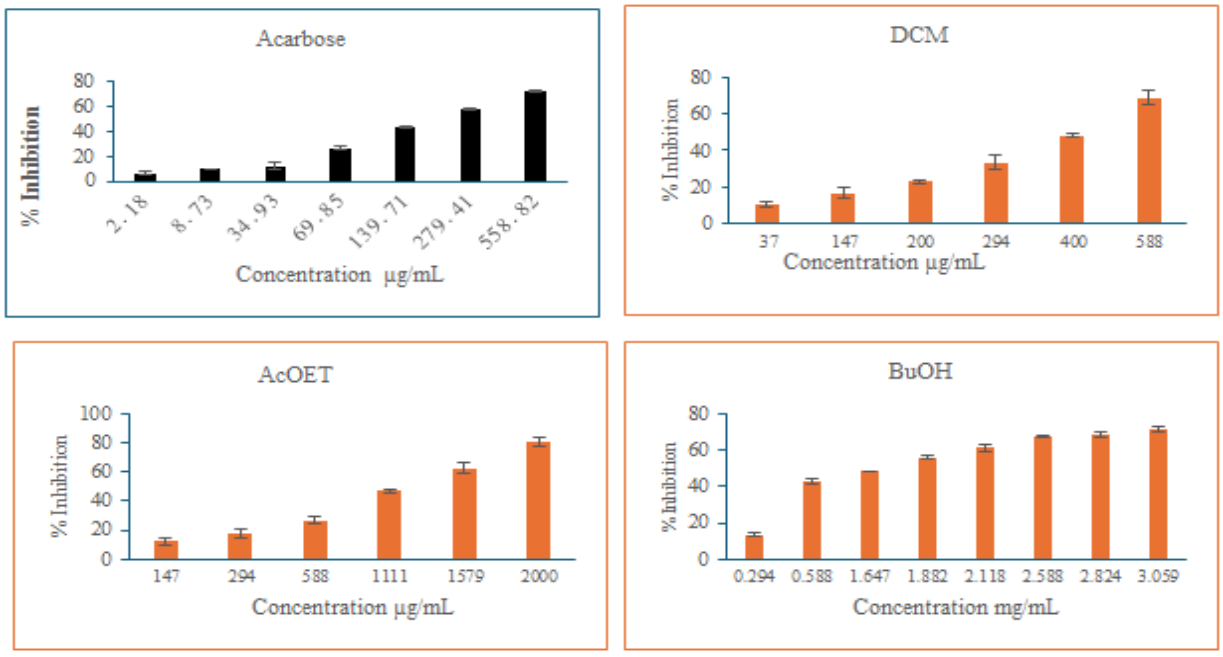

FIG. 7. **Inhibitory activity of *L. hastata* fractions and acarbose on *α-glucosidase*.**

As illustrated on **Figure 7**, all fractions exhibited a concentration-dependent effect on α-glucosidase inhibition. Indeed, it appears that α-glucosidase inhibition increases from 10 to 70% when the concentration of DCM fraction varies from 37 µg/mL to 588 µg/mL. For AcOET fraction, it varies from 12 to 80% in the range 147 µg/mL to 2000 µg/mL. In the case of BuOH fraction and for range concentration of 290 µg/mL to 3060 µg/mL, the inhibition rate of α-glucosidase varies from 13% to 71%. These results demonstrate that the different fractions of *L hastata* exhibit antidiabetic properties through inhibition of the α-glucosidase. The determination of IC_{50} of the different fractions is summarized in **TABLE 3**.

TABLE 3: **IC_{50} values of different fractions and acarbose.**

Plant	Fractions	IC_{50} (mg/mL)
L. hastata	DCM	0.431 ± 0.012
	AcOET	1.195 ± 0.004
	BuOH	1.623 ± 0.010

Standard	Acarbose	0.215 ± 0.004

One can see that the IC_{50} obtained is equal to 0.431 µg/mL ± 0.012 mg/mL, 1.195 µg/mL ± 0.004 mg/mL and 1.623 µg/mL ± 0.010 mg/mL for DCM, AcOET and BuOH respectively. The IC_{50} value of the standard acarbose is close to 0.215 µg/mL ± 0.004 mg/mL. In comparison with the value obtained by acarbose, DCM is the most active of fractions followed by AcOET and BuOH respectively.

A deep analysis of these results could be explained by the perfect correlation between the content of total polyphenol compounds (especially total flavonoids) and antidiabetic properties. These results are in agreement with the previous data reported in the literature [12, 20].

Relationship between the *α-glucosidase* inhibition and total phenolic content and total flavonoid content

According to the literature, the presence of bioactive compounds such as polyphenolic and flavonoids in plant extracts could confer inhibitory effects on the activity of α-glucosidase [23]. Previously studies on *Cassia occidentalis* and *Euphorbia hirta* showed similar results and a correlation between total phenolic and flavonoids content with antidiabetic activity is established [11, 24].

In this work, the R^2 correlation coefficients between phenolic compound contents, total flavonoids and α-glucosidase inhibition are being evaluated and the obtained results are presented in **FIGURE 8.**

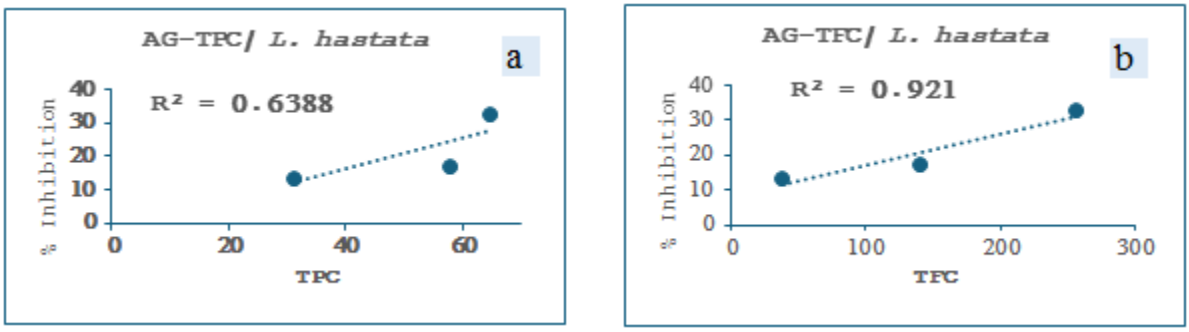

FIG. 8. **Correlation between total phenolic content (a) and total flavonoid content (b) and α-glucosidase inhibition in the different fractions of *L. hastate.***

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As indicated, the R^2 values vary between 0.639 and 0.921 for phenolic compounds and total flavonoids compounds respectively.

From these results, there is a positive correlation between these bioactive compounds and the inhibition of α-glucosidase indicating that antidiabetic effect is more affecting by flavonoids compounds than polyphenolic one. So, for *L. hastata*, phenolic compounds contributed to 63.88% of the antidiabetic activity while flavonoids contributed to 92.1%. These results corroborate those reported in the literature which established a good relationship between the antidiabetic properties of plant extracts with their phenolic compounds and flavonoids contents [9, 11].

Conclusion

Phytochemical screening of *L. hastata* shows that DCM, AcOET and BuOH fractions contain polyphenolic compounds such as flavonoids, tannins and quinones. Quercetin and rutin were identified in the ethyl acetate and BuOH fraction respectively. DCM and AcOET have the highest content of polyphenol respectively 64.5 µg EGA/mg and 57.5 µg EGA/mg of extract. The same fractions contain also more total flavonoids content estimated at 255.738 µg EQ/mg of extract for DCM and 139.683 µg EQ/mg of extract for AcOET. Fraction with high level of phenolic compounds showed best antidiabetic properties. DCM is the most active fraction with

IC_{50} =0.431 mg/mL ± 0.012 mg/mL followed by AcOET with IC_{50} =1.195 mg/mL ± 0.004 mg/mL. These results showed a correlation between this activity anti-diabetic activity and the level of polyphenolic and flavonoids present in each fraction.

In perspective, isolation and characterization of the active extracts will be done in order to determine the bioactive molecules responsible for this antidiabetic activity.

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Disclosure statement

The authors declared that they have no competing interests.

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