International Research Journal of Pure and Applied Chemistry

Volume 25, Issue 4, Page 44-50, 2024; Article no.IRJPAC.118449
ISSN: 2231-3443, NLM ID: 101647669

Anti-Diabetic Activities of the Extracts from *Euphorbia hirta* L. (Euphorbiaceae) Specie found in Burkina Faso Using α-Glucosidase Inhibitor

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/irjpac/2024/v25i4865

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/118449

Original Research Article

Received: 25/04/2024
Accepted: 20/06/2024
Published: 16/07/2024

ABSTRACT

Euphorbia hirta L. is an herbaceous plant used in traditional medicine to treat several diseases. In the present work, different fractions of *Euphorbia hirta* L. hydroalcoholic extract were investigated for their anti-diabetic properties on α-glucosidase's inhibition activities. Chemical screening realized

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Cite as: *Karanga, Yssouf, Têeda Hamidou Ganamé, Wende-Konté Hazael Conania Nikiema, and Issa Tapsoba. 2024. "Anti-Diabetic Activities of the Extracts from Euphorbia Hirta L. (Euphorbiaceae) Specie Found in Burkina Faso Using α-Glucosidase Inhibitor". International Research Journal of Pure and Applied Chemistry 25 (4):44-50. https://doi.org/10.9734/irjpac/2024/v25i4865.*

following Folin-Ciocalteu and $AlCl_3$ methods showed that ethyl acetate (AcOET) and butanol (BuOH) extracts contain 432.687 ± 1.832 µg and 332.433 ± 1.255 µg GAE/mg of extract for phenolic compounds. The same extracts contained high levels of total flavonoids estimated at 417.850 ± 15.941 µg and 227.640 ± 2.657 µg QE/mg of extract respectively. The anti-diabetic activities revealed that AcOET and BuOH have a very significant inhibitory potential on the enzymatic activity of α-glucosidase with an IC_{50} values of 1.295 ± 0.035 µg/mL and 2.188 ± 0.204 µg/mL respectively compared to acarbose (IC_{50} = 214.825 ± 3.816 µg/mL).

Keywords: Euphorbia hirta L; α-glucosidase; diabetes; flavonoids; inhibition.

1. INTRODUCTION

Diabetes, well-known as an ancient disease, is characterized by a disordered metabolism of carbohydrates linked to a total or relative insufficiency of the action of insulin [1]. International Diabetes Federation (IDF) statistics for 2021 indicated that 537 million adults were living with diabetes worldwide, with 6.7 million deaths in the same year [2]. Disruption of insulin activity results in permanent hyperglycaemia, with fasting blood glucose levels of 1.26 g/L or more than 2 g/L. In addition, many studies have shown that oxidative stress contributes to the development and progression of several diseases, including diabetes [3,4]. People with diabetes often suffer from end-stage renal failure, blindness, and lower-limb amputations. Type 2 diabetes was responsible for 416,000 deaths on the continent in 2021 [5]. In Burkina Faso, the prevalence of diabetes is 4.9% nationally and 13.9% in urban areas [6]. Despite modern medicine progress, the therapeutic management of diabetics remains a major problem for healthcare systems. Regular use of antidiabetic drugs such as insulin and oral hypoglycaemic leads to undesirable side effects [7,8]. Recently, diabetologists have concluded that a therapeutic complement consisting of plant extracts is necessary to optimize the treatment of diabetes [9,10]. Most of these plants contain high levels of phenolic compounds, particularly flavonoids, with proven pharmacological properties. One of these plants is *Euphorbia hirta* L. (*E. hirta* L.), a medicinal plant acclimatized in Burkina Faso. *E. hirta* L. is well-known to contain phenolic compounds [11]**.** Previous studies have shown that the presence of bioactive compounds could inhibit the enzymatic activity of alpha-glucosidase [12-14] and therefore regulate blood glucose levels. The present work focused on assessing the antidiabetic properties of fractions enriched with phenolic compounds from *E. hirta* L. using the in vitro model.

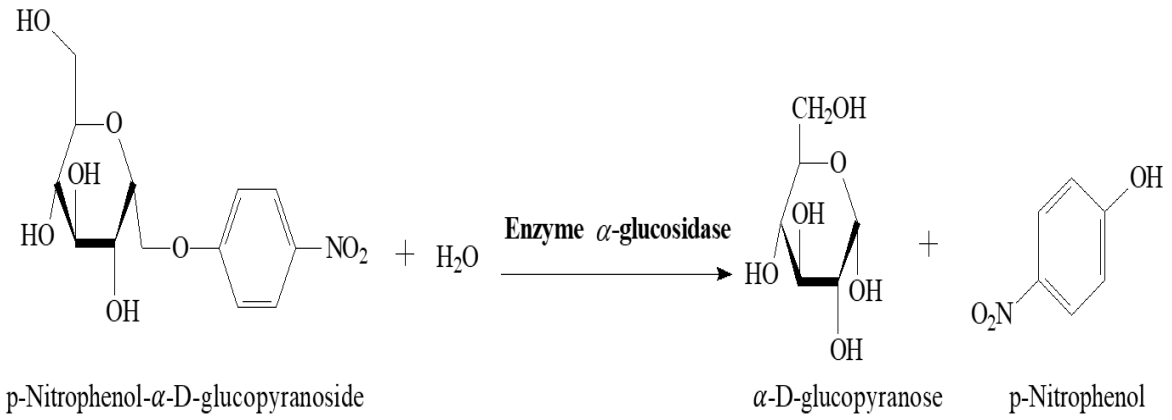
2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

The leafy stems of *E. hirta* L. were collected in August 2019 in Ouagadougou, the capital of Burkina Faso, at GPS coordinates 12°18'22.77822" N; 1°30'10.34362" W. The plant species were identified by a botanist of the University Joseph KI-ZERBO. The plant material was washed carefully and dried at room temperature. The dry matter was transformed into a fine powder using an electric grinder. Finally, extraction was carried out according to a protocol described in the laboratory [15]**,** with a few modifications. Three (03) extracts were prepared: DCM: dichloromethane fraction**;** AcOET: ethyl acetate fraction; BuOH: butanol fraction.

2.2 Determination of Total Phenolic Compounds

The total phenolic compound (TPC) content of the different fractions was determined by the Folin-Ciocalteu method. Indeed, the Folin-Ciocalteu Reagent (FCR) is reduced to tungsten oxide and molybdenum by the phenolic compounds. The intensity of the blue color of the molybdenum is correlated to the quantity of phenolic compounds contained in the sample [16]. 60 µL of the sample at different concentrations was added to 60 µL of FCR. After vigorous stirring and allowing the mixture to stand for 8 min, 120 µL of 7.5% (w/v) Na_2CO_3 was added. The reaction mixture was then incubated at laboratory temperature (25°C) for 30 min. Absorbances were read at 760 nm using a spectrophotometer (SPECTROTOstar NANO, BMG LABTECH, Ortenberg, Germany). The phenolic compound content was calculated by using calibration curve established gallic acid with between established and gallic acid. These values are expressed in micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg of extract).



Scheme 1. Hydrolysis of pNPG by α-glucosidase to release glucose and pNP

2.3 Determination of Total Flavonoids

The total flavonoids contained in the different fractions of *E. hirta* L. were quantified by a colorimetric method described in the literature with slight modifications [17]. For this, 50 µL of sample at different concentrations was added to 150 µL of distilled water followed by 15 µL of $NaNO_2$ at 5% (w/v). 5 min later, 15 µL of $AlCl_3$ 10% (w/v) was added, followed by incubation at room temperature for 6 min. Absorbance at 510 nm were taken after the addition of 50 µL of 1 N NaOH. A quercetin calibration curve was established and used to deduce the flavonoid content, expressed as micrograms of quercetin equivalent per milligram extract (µg QE/mg of extract).

2.4 Assessment of Antidiabetic Activity

The antidiabetic activity of the different fractions extracts of *E. hirta* L. was assessed using the chromogenic method reported by Ranilla with slight modifications [18]. To do this, we used α-glucosidase, which is a digestive enzyme that converts carbohydrates into glucose according to the following Scheme 1.

Inhibition of the activity of this enzyme leads to a decrease of the amount of glucose in blood and this reaction has been reported as a therapeutic alternative in the treatment of hyperglycaemia [19]. The anti-diabetic activity of the different extracts was tested in 96-well plates with a maximum reaction mixture volume of 250 µL per well. The reaction was started with a mixture of 20 µL of α-glucosidase enzyme (1 unit/mL), 120 µL of phosphate buffer (0.1 M; pH 6.9), and 10 µL of the extract at different concentrations. After 15 min pre-incubation at 37°C, the enzymatic reaction was initiated by adding 20 µL of p-nitrophenyl-α-D-glucopyranoside (5 mM prepared in 0.1 M phosphate buffer; pH 6.9). The reaction mixture was incubated again for 15 min at 37°C. At the end, 80 µL of sodium carbonate solution (0.2 M) was added to the reaction mixture. The inhibitory activity of the α-glucosidase enzyme was determined by reading the absorbance of p-nitrophenol released from p-nitrophenyl-α-D-glucopyranoside at 405 nm using a spectrophotometer (SPECTROTOstar NANO, BMG LABTECH, Ortenberg, Germany). The reaction system without plant extracts was used as a control and the system without the α-glucosidase enzyme was used as a blank to correct for background absorbance. The percentage inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = (\text{Control absorbance} - \text{Sample absorbance}) / (\text{Control absorbance}) \times 100$$

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Compound and Flavonoid Contents

The contents of total phenolic compounds (TPC) and total flavonoids (TFC) in the different fractions of *Euphorbia hirta* L extract were estimated using the equations of the calibration curves for gallic acid ($y = 88.3090x + 0.0473$; $R^2 = 0.9991$) and quercetin ($y = 4.8668x + 0.0162$; $R^2 = 0.999$) respectively. The results obtained are shown in Table 1.

Table 1. Total phenolic compound and flavonoid contents of different fractions of *E. hirta* L.

	***E. hirta* L. fractions**		
	DCM	**AcOEt**	**BuOH**
TPC (µg GAE/mg)	142.312 ±2.214	432.687 ± 1.832	332.433 ± 1.255
TFC (µg QE/mg)	144.729 ±3.043	417.850 ± 15.941	227.640 ± 2.657

Table 2. IC_{50} (µg/mL) values of the antidiabetic activity of different fractions of *E. hirta* L. and acarbose (used as positive control)

IC_{50} (µg/mL)		
***E. hirta* L. fractions**		**Standard**
AcOET	**BuOH**	**Acarbose**
1.295 ± 0.035	2.188 ± 0.204	214.825 ± 3.816

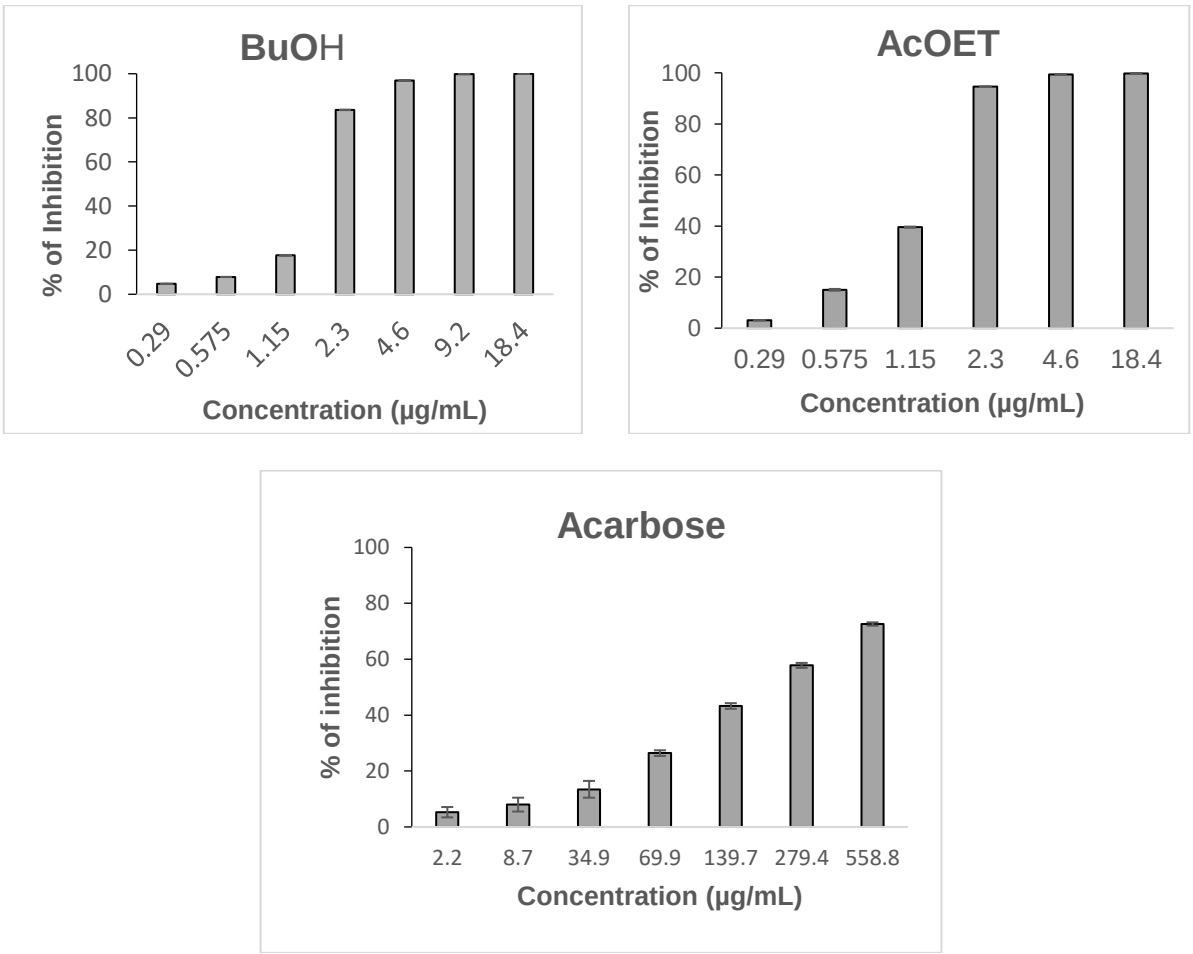


Fig. 1. Inhibitory activity of different fractions of *E. hirta* L. and Acarbose (used as positive control) on α-glucosidase enzyme

Further analysis of Table 1 shows that the highest levels of the phenolic compounds estimated are equal to 432.687 µg and 332.433 µg GAE/mg of extract respectively for AcOEt and BuOH fractions. DCM fraction contains the lowest amount which is close to 142.312 µg GAE/mg of extract. These strong phenolic compounds contents obtained for AcOET and BuOH fractions are in agreement with the literature and show that *E. hirta* L. is a plant rich in phenolic compounds [20]. The total flavonoids are evaluated to 417.850 µg and 227.640 µg QE/mg of extract for AcOET and BuOH respectively. However, the lowest TFC contents

is observed in DCM fraction. These results show that the content of phenolic compounds or total flavonoids varies considerably according to the polarity of extraction solvents. The presence of non negligeable content of phenolic compounds particularly flavonoids in DCM reveals that dichloromethane would also extract apolar compounds in addition to flavonoid aglycones. This result agrees with the literature [15,21]. Moreover, the total phenolic compounds positively correlated with total flavonoids means which the higher the total phenolic content, the higher the total flavonoid contents.

3.2 Antidiabetic Activity

The antidiabetic tests were carried out on the fractions containing the highest levels of phenolic compounds and total flavonoids, in particular AcOEt and BuOH, and using acarbose as a positive control. The obtained results are shown in Fig. 1.

Analysis of the histograms in Fig. 1 reveals that both fractions AcOEt and BuOH exhibit a highly significant inhibitory effect on α-glucosidase enzyme activity. This inhibitory activity is highly dose dependent. For the AcOEt fraction, the percentage of inhibition increased proportionally with the dose used, reaching 95% for a concentration of 2.3 µg/mL. The enzyme is completely inhibited for a concentration 4.2 µg/mL of AcOET.

For the BuOH fraction, a percentage inhibition is equal to 83.61% for a concentration of 2.3 µg/mL. This percentage inhibition reaches 100% for concentration above 9.2 µg/mL.

These data demonstrate that AcOET fraction presented more pronounced inhibition effect on α-glucosidase than BuOH and are in correlation with phenolic and flavonoids contents. As discussed above, that ethyl acetate fraction contains the highest level of phenolic and flavonoids compounds than butanol fraction.

In addition, the antidiabetic effect obtained with the standard acarbose revealed it is less active than acetate and butanol fractions. Indeed, one can see in the histogram that the inhibition rate is close to 84% for a concentration of acarbose of 558.82 µg/mL. In the case of butanol fraction, 9.2 µg/mL is necessary to reach a complete inhibition rate of α-glucosidase enzyme. For acetate fraction, only 4.2 µg/mL is necessary for a complete inhibition. These result show that *E. hirta L.* extracts are more antidiabetic than the reference acarbose. Previous authors obtained similar results during their work which focused on the evaluation of the antidiabetic properties of medicinal plant extracts [21,22]. To confirm all the obtained results, the IC_{50} values (µg/mL) for all the different fraction was calculated and summarised in Table 2.

A detailed analysis of these results confirms that the butanol and acetate fractions with the IC_{50} values of 2.188 and 1.295 µg/mL respectively are more active on the α-glucosidase enzyme than the reference antidiabetic compound with an IC_{50} of 214.825 µg/mL. As mentioned previously, the lower value of IC_{50} exhibiting the antidiabetic properties of *Euphorbia hirta* L. fractions is correlated with the high amount of phenolic compounds. Indeed, in previous study, we highlighted the presence of quercetin, quercitrin, isoquercetin, myricitrin, myricetin-3-O-glucoside, myricetin-7-O-glucoside, kaempferol-3-O-glucoside, vitexin, and isovitexin in acetate fraction of *Euphorpbia hirta* L. [23]. These results are in agreement with those reported in the literature [24,25]. Indeed, it is well-known and reported that the antidiabetic properties of a plant extract are attributable to the phenolic compounds such as flavonoids [26]**.** Moreover, it has also reported been in the literature that quercetin and its derivatives have hypoglycaemic properties [27-29].

4. CONCLUSION

This study assessed the anti-diabetic properties of extracts enriched with phenolic compounds from *E. hirta* L.*,* a medicinal plant acclimatized in Burkina Faso. The results obtained document a high antidiabetic activity of ethyl acetate and butanol extracts with IC_{50} values of 1.295 ± 0.035 µg/mL and 2.188 ± 0.204 µg/mL respectively. This activity was even more pronounced the greater the number of phenolic compounds, particularly flavonoids, contained in the extract. These results therefore suggest the use of *E. hirta* L. extracts in the formulation of antidiabetic phytomedicines.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGMENTS

The authors acknowledge the ANEC/ISP (African Network of Electroanalytical Chemists/International Science Program), for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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