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Antioxidant and Anti-Hyperglycemic Activities of Aqueous and Hydro-Ethanolic Extracts of *Excoecaria grahamii* (Euphorbiacea)

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Abstract | **Original Research Article**

Background: *Excoecaria grahamii* is a traditional medicinal plant use in Burkina Faso and other part in the word to treat many diseases. However, its antioxydant and antidiabetic effects are not yet demonstrated. ***Objective:*** The aim of this study was to investigate the potential inhibitory effects of aqueous and ethanolic extracts of leaves and roots of *Excoecaria grahamii* on oxidative stress and hyperglycemia. ***Materials and Method:*** The antioxidant effect was investigated by FRAP, DPPH and ABTS essays while the anti-hyperglycemic effect were evaluated using α-amylase and β-galactosidase inhibitory assays. In addition, the phytochemical screening for some targeted compounds was carried out. ***Results:*** The result has shown that *Excoecaria grahamii* have antioxidant effect. Regarding the antidiabetic effect our data have shown only an inhibitory effect on α-amylase enzyme but not on β-galactosidase. Phytochemical study has shown the presence of gallic tannins, terpenoids and flavonoids in all the extract. Only aqueous extract have shown saponins. No catechic tannins and anthraquinones have been showed. ***Conclusions:*** This study suggests that *Excoecaria grahamii* contain chemicals components that can reduce oxidative stress and post prandial glucose enhance. It can be useful in the fight against antioxidant-related diseases such as those seen during ageing.

Keywords: *Excoecaria grahamii*, antioxidant, anti-hyperglycemia, α-amylase, β-galactosidase.

INTRODUCTION

Diabetes is one of the main causes of consultations and hospitalization in Burkina Faso among metabolic diseases according to the Health Statistical Yearbook [1]. This disease is more common in adults. It is known that excessive postprandial glucose excursions are a risk factor for developing diabetes mellitus [2]. This disease can also result from a complication of oxidative stress. To control diabetes, one of the interesting approaches is to limit the action of digestive enzymes such as α-amylase and β-glucosidase which activities increase blood glucose level [3].

Plants used in traditional medicine to treat diabetes generally contain phenolic compounds. These compounds constitute one of the most numerous and widely distributed group of substances in the plant kingdom, with more than 8000 phenolic structures currently known [4]. Polyphenols are known to protect the body against the release of peroxidized fatty acids. They can trap free radicals which are cytotoxic substances. Polyphenols that are considered antioxidants can prevent oxidative stress and reduce vascular complications related to diabetes. The presence of polyphenols in *Excoecaria grahamii* leaves extracts has been demonstrated [5, 6]. This plant is used in traditional medicine for many therapeutic virtues. However, his effects related to diabetes and oxidative stress is not yet documented.

The aim of this work was to evaluate the antioxidant and anti-hyperglycemic potential activities of *Excoecaria grahamii*.

MATERIALS AND METHODS

Plant Material and Extracts Preparations

The plant sample was collected and aqueous *Excoecaria grahamii* made as describe in our previous article [7]. The plant sample was collected from its natural habitat in the locality of Kombissiri in the south-eastern region of Burkina Faso, located at 42 km from Ouagadougou the capital of Burkina Faso (11°55′ 33.8″ North; 01°17′10″ East). This sample was collected during the dry season and identified by the Herbarium of the Biodiversity Centre of University Joseph KI-ZERBO, where the voucher specimen (ID No: 16703 and sample No: 6786) was stored. The leaves of the plant were dried in shade at room temperature (30 ± 2 ° C) for 14 days.

For producing aqueous extract of *Excoecaria grahamii*, the leaves were powdered and macerated using 100 g of powder in one liter (1 L) of distilled water for 24 h. The maceration was done using a magnetic stirrer, and the solution was then filtered using a Whatman No. 2 filter paper and freeze-dried. The powder obtained was yellowish in colour. It was stored at-4°C and used for the different tests. The extraction yield was approximately 13% (w/w).

Aqueous extract was made only from dried leaves (AEL) while hydro-ethanolic extracts were made from dried leaves and roots. Hydro-ethanolic extracts were prepared using 500 g of leaves and 46.50 g of roots powders that were mixed with 1250 mL and 250 mL of hydro-ethanolic solutions 80% v/v, respectively. The mixtures were macerated at laboratory room temperature for 48 h. After maceration, the extracts were leached in a column until exhausted using a percolator. The extract solutions obtained were first concentrated under reduced pressure in the rotavapor (water bath temperature 50°C) and then dehydrated for 72 hours in crystallizers placed in a ventilated oven preset at 45-50°C. The average extraction yields are of the order of 28.90 and 9.10 % respectively for ethanolic leaves extract and ethanolic roots extract.

Phytochemical Analysis

Test for Tannins

Search for catechic tannins is made from reagent Stiasny. 5 ml of each extract were evaporated to dryness. After adding 15 mL of reagent Stiasny the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterized catechic tannins [8].

For gallic tannins, about 0.5 mL of extracts was stirred with 10 mL distilled water. Production of a blue, blue-black, green or blue-green coloration or precipitate on the addition of $FeCl_3$ (1%) reagent was taken as evidence for the presence of tannins [9].

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to small amount of extract. Immediate development of a red color indicated the presence of flavonoids [9].

Test for Saponins

About 0.5 mL of extract was shaken vigorously with water in a test tube. If a frothing was produced and it was stable for 1-2 minutes and persisted on warming, it was taken as preliminary evidence of saponins.

Test for Anthraquinones

About 1g of sample was mixed with 10mL of chloroform. The mixture was heated in a water bath for 15min, then filtered and made up to 10mL with chloroform. In another test tube, 1mL of chloroform extract and 1mL of 10% aqueous KOH was mixed and the presence of the anthraquinones is confirmed by the development of a red color [10].

Test for Terpenoids

Two (2) mL of the organic extract was dissolved in 2 mL of chloroform and evaporated to dryness 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids [11].

Antioxidant Activities

Ferric Reducing/Antioxidant Power (FRAP) Assay

The method used is that described by Sayah *et al.*, (2017) and based on Oyaizu (1986) method [12, 13]. Briefly, the extract (1 mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of trichloride iron ($FeCl_3$) solution (0.1%). The absorbance was measured at 700 nm with a spectrophotometer (JENWAY 6305). Increased absorbance values indicate a higher reducing power. The results were expressed as ascorbic acid equivalent per gram of extract dry weight (mg AAE/g edw). The ascorbic acid calibration curve was $y = 0.005 * X + 0.163$; $r^2 = 0.994$.

DPPH Radical Scavenging Assay

To perform this test, a solution (0.5 mL) of 2.2-diphenyl-1-picrylhydrazyl (DPPH, 0.2 mM) was mixed with 2.5 mL of trolox (0.33, 0.66, 1, 2, 2.66, 3.33, 4 and 4.66 µg / mL) or extract (0.42 - 41.17 µg / mL). After 30 min in the dark at room temperature, the absorbance was measured at 517 nm by a spectrophotometer (JENWAY 6305) from the calibration curve ($y = 13.82 * X + 23.48$) of trolox (6-hydroxy acid -2, 5, 7, 8-tetramethylchroman-2-carboxylic acid). All the experiment was carried up triplicate. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The

percentage of inhibition was calculated according to the following formula:

$$= \frac{A_0 - A_1}{A_0} \times 100$$

DPPH scavenging activity:
A_0 = Absorbance control.
A_1 = Absorbance of the Sample solution or standard.

Scavenging activity in this assay was expressed as IC_{50}, which represents the concentration of the extract required to inhibit 50% of the free radical scavenging activity.

ABTS Radical Scavenging Assay

The ability of our extracts to scavenge the ABTS radical was determined according to the previously described method [13, 14]. A solution of ABTS radical cation ($ABTS^{\bullet+}$) was prepared by the reaction between 10 mL of 2 mM ABTS in H_2O and 100 μL of 70 mM potassium persulphate at room temperature in the dark for 24 h. The $ABTS^{\bullet+}$ solution was then diluted with methanol to obtain absorbance of 0.70 at 734 nm. Samples were prepared in triplicate by diluting 200 μL of extracts (0.1mg/mL) in 2 mL of the $ABTS^{\bullet+}$ solution diluted with methanol and allowed to react for 1 min. The absorbance was recorded on a spectrophotometer at 734 nm. The antioxidant activities of samples were expressed as TEAC (Trolox equivalent antioxidant capacity) values, defined as the concentration of standard Trolox with the same antioxidant capacity of the extract under investigation. The results were represented as Trolox equivalent per gram of extract dry weight (mg TE/g edw).

Antidiabetic Activity

α-Amylase Inhibitory Assay

The α-amylase inhibitory potentials were investigated by reacting different concentrations of the extracts with α-amylase enzyme and starch solution, according to the previously described method [15] with slight modifications. A mixture of 250 μL of samples and 250 μL of 0.02 M sodium phosphate buffer (pH = 6.9) containing the enzyme α- amylase (240 U/mL) was incubated at 37 °C for 20 min. Then, 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.9) was added to the reacting mixture. Therefore, the reaction mixture was incubated at 37 °C for 15 min. Thereafter, 1 mL of dinitrosalicylic acid (DNS) was added, and the reaction mixture was incubated in a boiling water bath for 10 min. Then, the reaction mixture was diluted by adding 2 mL of distilled water, and absorbance was measured at 540 nm in the spectrophotometer (JENWAY 6305). Acarbose was used as positive control. The results were expressed as percentage inhibition and calculated using the following formula:

Inhibition (%) =

$$\frac{[(A_c - A_{cb}) - (A_s - A_{sb})]*100}{(A_c - A_{cb})}$$

In this formula, $\boldsymbol{A_c}$ refers to the absorbance of control (enzyme and buffer); $\boldsymbol{A_{cb}}$ refers to the absorbance of control blank (buffer without enzyme); $\boldsymbol{A_s}$ refers to the absorbance of sample (enzyme and inhibitor); and $\boldsymbol{A_{sb}}$ is the absorbance of sample blank (inhibitor without enzyme). Moreover, IC_{50} values (concentration of inhibitor required to inhibit 50 % of enzyme activity) were determined.

β-Galactosidase Inhibitory Assay

The β-galactosidase inhibitory activity was assessed as describe by Omari *et al*., 2019 [16] using 2-Nitrophenyl-β-D-galactopyranoside as substrate, which is hydrolyzed by β- galactosidase to release 2-nitrophenyl. Briefly, a mixture of 150 µL of the samples at different concentrations (0.5- 5mg/mL) and 100 µL of sodium phosphate buffer 0.1M (pH=7.6) containing the enzyme β-galactosidase solution (0.1U/mL) was incubated at 37°C for 10 min. After that, 200 µL of 2-Nitrophenyl-β-D-galactopyranoside solution 1mM in sodium phosphate buffer 0.1M (pH=7.6) was added. The reaction mixtures were incubated at 37°C for 30min. After incubation, 1mL of 0.1M of Na_2CO_3 was added to stop the reaction and the absorbance was recorded at 410 nm using the spectrophotometer. The β-Galactosidase inhibitory activity was expressed as percentage inhibition and calculated using the same formula as the α-amylase test, and the IC_{50} values were determined. Quercetin was used as positive control and the experiment was carried out in triplicate.

Reagents

Potassium ferricyanide, trichloroacetic acid, potassium persulphate, trichloride iron, dinitrosalicylic acid (DNS), 2-Nitrophenyl-β-D-galactopyranoside, α-amylase from *Bacillus licheniformis*, β-galactosidase from *Aspergillus oryzae*, acarbose, DPPH, ABTS, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and ascorbic acid were purchased from Sigma-Aldrich (France). All the reagents used were of analytical grade.

Statistical Analysis

The data were analyzed using GraphPad Prism 5.03 and the results are expressed as mean ± standard error of the mean. The same software was used to determine IC_{50} values using non-regression curve. Differences between the means were determined by one way analysis of variance (one-way ANOVA). Post hoc procedure was used for significance of difference. A difference in mean values of $p < 0.05$ was considered to be statistically significant.

RESULTS

Antioxidant Activity

The antioxidant capacity assays used to assess these extracts are FRAP, DPPH, and ABTS. Through these methods, all of our extracts showed antioxidant activities.

In FRAP method, our results showed that the aqueous leaves extract is the most active (620.00 ± 2.83 mg AAE/g of extract) compared to ethanolic leaves extract (586.93 ± 0.35 mg AAE/g of extract) and ethanolic roots extract (508.13 ± 6.07 mg AAE/g). In ABTS method ethanolic leaves extract is the most active (56.30 ± 0.09 mg TE/g edw) compared to aqueous leaves extract (55.69 ± 0.12 mg TE/g edw) and ethanolic roots extract (56.08 ± 0.03 mg TE/g edw) (Table 1).

Table 1: Antioxidant activities measurement by FRAP and ABTS methods

Extracts	FRAP (mg AAE/g edw)	ABTS (mg TE/g edw)
Ethanolic roots extract	508.13 ± 6.07[a]	56.08 ± 0.03[a]
Aqueous leaves extract	620.00 ± 2.83[c]	55.69 ± 0.12[a]
Ethanolic leaves extract	586.93 ± 0.35[b]	56.30 ± 0.09[b]

mg AAE/g edw: mg of ascorbic acid equivalent per gram of extracts dry weight
mg TE/g edw: mg of Trolox equivalent per gram of extracts dry weight)
Values in the same column not sharing a common letter (a to c) differ significantly, $p < 0.05$

In DPPH method, IC_{50} measured showed that ethanolic leaves extract (ELE) is more active than aqueous leaves extract (ALE) and ethanolic roots extract (ERE). The IC_{50} values of these measures are 1.74, 2.25 and 7.80 µg/mL respectively (figure 1).

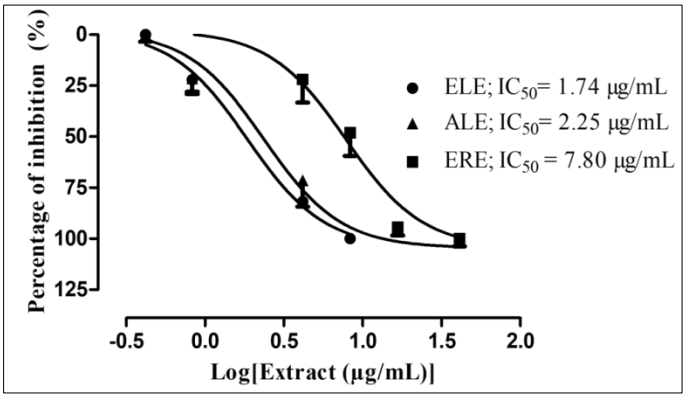

Figure 1: DPPH concentration-response curves

Phytochemical Screening

Phytochemical screening revealed the presence of gallic tannins, terpenoids and flavonoids in all our extracts, but saponins were only found in the aqueous leaf extract. The presence of anthraquinones was not revealed in all the extracts (Table 2).

Table 2: Phytochemical screening

Compounds	ELE	ERE	ALE
Gallic tannins	+	+	+
Catechic tannins	-	-	-
Saponins	-	-	+
Terpenoids	+	+	+
Anthraquinones	-	-	-
Flavonoids	+	+	+

(+): Indicates positive test result; (-): Indicates negative test result
ELE: Ethanolic Leaves Extract of *Excoecaria grahamii*
ERE: Ethanolic Roots Extract of *Excoecaria grahamii*
ALE: Aqueous Leaves Extract of *Excoecaria grahamii*

Antidiabetic Activities

The aqueous and ethanolic extracts of *Excoecaria grahamii* (ALE, ELF, ERE) inhibit the activity of α-amylase. The inhibition activity is more important for ELE. This extract achieved 80% inhibition at the concentration of 4mg/mL. About ALE the percentage of inhibition reached 80% at the concentration of 8mg/mL. At the same concentration of

8 mg/mL the inhibition of α-amylase produced by ERE remains below 50% (Figure 2). The values of IC_{50} measured for ELE, ALE and ERE are approximately 3.20, 5.80 and >8 mg/mL respectively.

Regarding β-galactosidase inhibition, all the extracts are not active up to the concentration of 8 mg/mL.

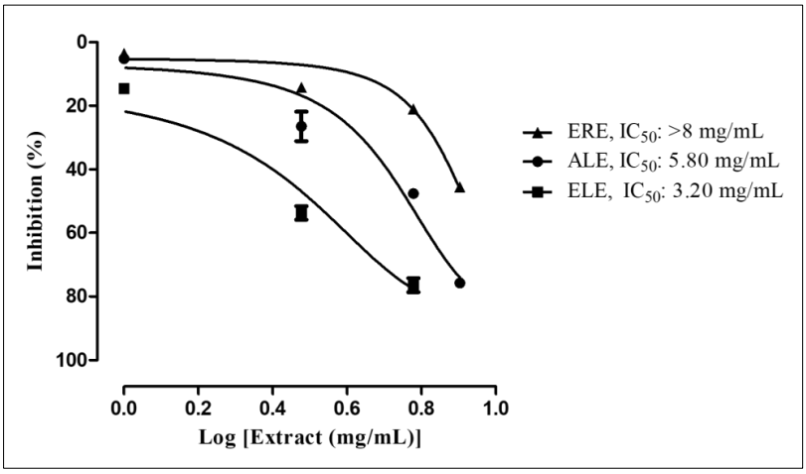

Figure 2: Effect of aqueous extract (ALE) and ethanolic extracts of leaves (ELE) and roots (ERE) on the enzymatic activity of α-amylase

DISCUSSION

Many radicals and reactive oxygen species (ROS) are constantly formed in human body. They are implicated in many diseases such as diabetes. Medical plant which is potential source of antioxidant can be an important alternative way to limit their negative impact on the body physiology. In this work our data indicate that, *Excoecaria grahamii* aqueous and ethanolic extracts have antioxidant activities relate to the different methods that were used (FRAP, DPPH, and ABTS essays). But the extracts did not follow the same ranking in the different essays. This is probably due to the different mechanisms of action involved in each assay or the composition of the extract. About the composition of the extract, it can depend on the part of the plant used or the type of solvent used to perform the extractions. Leaves and roots are the plant parts that were used for this study. We can highlight that the leaves part seem to have the best antioxidant activity because through all the tests of the study, the leaves extracts were the most effective. These data are in agreement with the fact that the aerial part of the plant is the most used in the traditional medicine for the care [17]. Furthermore, it is well know that FRAP mechanism of action is different from those of ABTS and DPPH [18, 19]. For instance, DPPH and ABTS assays are based on electron and H atom transfer, while the FRAP assay is based on electron transfer reaction [18]. Each method used a particular free radical showing that our extracts could have the ability to scavenge different kinds of free radicals under oxidative stress condition. This could be an advantage because, in the human body, many kind of free radicals such as superoxide, hydroxyl and lipid peroxidation are produced to increase oxidative stress particularly when ageing [20, 21]. Regarding the anti-diabetic activity, β-galactosidase and α-amylase are enzymes which can be encountered in the human body. β-galactosidase is a membrane-bound enzyme located in the brush border epithelial cells of the small intestine while α-amylase is produced and secreted by salivary glands and by the pancreas. In the intestine, these enzymes are responsible for the breakdown of amylose and lactose respectively for α-amylase and β- galactosidase into disaccharides such as maltose and monosaccharides such as glucose and galactose [22, 23]. Maltose is a final product of starch digestion by α-amylase, and consists of 2 glucose units linked by an α-1,4 bond while glucose and galactose are the final products of β-galactosidase action resulting by hydrolyzing of β-1,4-D-galactosidic linkages [2, 22]. The consequence of these enzymes actions is an increase of post-prandial blood glucose level. So, the management of type 2 diabetes mellitus could be done both by reducing oxidative stress as well as by delaying the absorption of monosaccharides through the inhibition of the carbohydrates-hydrolyzing enzymes. In this work, our data indicated that all the extracts have an inhibitory effect on α-amylase but not on β-galactosidase. These antidiabetic effects of the extracts suggest that *Excoecaria grahamii* contain some compounds whose effects can be compared to those of acarbose, an antidiabetic drug with a known mechanism of action to inhibit α-amylase [24]. However, the IC_{50} obtained in this anti-hyperglycemic experiment are very high compared to that of acarbose found by Hashim *et al.*, (2013). Nevertheless, our results suggest that the extracts could biologically reduce glucose absorption in the gut by limiting the starch transformation. In this case *Excoecaria grahamii*, particularly the aqueous leaves extract contain some compounds that could be an oral antihyperglycemic agent again type 2 diabetes mellitus.

In addition, the antioxidant and antidiabetic activities of *Excoecaria grahamii* are comparable to those of the most widely reported species of the genus *Excoecaria* named *Excoecaria agallocha* [25]. All the extracts activities highlighted in this work are probably based on their chemical contents [26]. The phytochemical screening has shown the presence of flavonoids, gallic tannins and terpenoids in all the extracts and only saponosides in aqueous extract. These secondary metabolites may be the main compounds of the plant which provided the antioxidant and antidiabetic activities [27].

CONCLUSION

The aim of this work was to highlight the antioxidant and anti-hyperglycemic potential activities of *Excoecaria grahamii*. The results have shown that aqueous and ethanolic leaves extracts and ethanolic extract of roots have antioxidant activities in the FRAP, DPPH and ABTS assays. Likewise, the three extracts have a slight anti- hyperglycemic effect on α-amylase but not on β-galactosidase. The leaves extracts seem to present the most efficient activities. About the phytochemical compounds, the presence of flavonoids, gallic tannins, and terpenoids have been shown in all the extracts and only saponins in the aqueous extract. The results suggest that *Excoecaria grahamii* could be a potential antioxidant to fight oxidative stress and related diseases; it could not be used as an anti-enzymatic agent oriented on amylase in order to fight type 2 diabetes but the results need to be deepening.

CONSENT FOR PUBLICATION

All authors read and approved the final content of this manuscript for publication.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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