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# Development of a Thin-Layer Chromatography-Enzymatic Test Combination Method for the Isolation of $\alpha$ -Glucosidase Inhibitors From *Thymelaea hirsuta*

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## Abstract

A rapid, easy and simple method for the isolation and purification of  $\alpha$ -glucosidase inhibitors of the ethyl acetate extract of *Thymelaea hirsuta* (EaTh) by a combination of thin layer chromatography (TLC) and enzymatic test has been developed. EaTh was demonstrated previously a potent  $\alpha$ -glucosidase inhibitory effect. In this study, we developed a simple TLC-enzymatic test (TLC/EZ) combination to isolate  $\alpha$ -glucosidase inhibitors present in EaTh. EaTh was extracted by Soxhlet from *Thymelaea hirsuta* (*T. hirsuta*). The EaTh was separated on a silica gel column and then on a TLC plate. After TLC separation, the TLC/EZ combination method was applied.  $\alpha$ -glucosidase inhibitors were detected directly in the TLC plate using the glucose oxidase peroxidase method (GOD-POD). A good detection of active compounds was obtained in the TLC favoring the TLC/EZ method. Active compounds were then characterized using high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The main  $\alpha$ -glucosidase inhibitors present in EaTh have a molecular ion  $[M + H]^+$  at  $m/z = 543$ . This proposed method is suitable for a reliable isolation and purification of  $\alpha$ -glucosidase inhibitors present in EaTh. It could be proposed as an interesting alternative of the classical method for the isolation and purification of  $\alpha$ -glucosidase inhibitors in plant extracts.

**Keywords:**  $\alpha$ -glucosidase inhibitor, thin-layer chromatography, HPLC-MS, glucose oxidase peroxidase method, *T. hirsuta*

## Highlights

- New thin layer chromatography-enzymatic test combination method for  $\alpha$ -glucosidase inhibitors isolation from *Thymelaea hirsuta*.
- In classical approach, chromatographic methods alone need a long chain process to isolate and purify bioactive compounds.
- Combination of thin layer chromatography and enzymatic tests is an excellent alternative to isolate and purify  $\alpha$ -glucosidase inhibitors from plants.
- New combine approach is a time and energy gain in isolating plant compounds against  $\alpha$ -glucosidase.

## Introduction

This disclosure is concerned generally with  $\alpha$ -glucosidase inhibitors isolation and purification from active plant extract (against  $\alpha$ -glucosidase) using a rapid and simple combination approach of TLC methods and enzymatic tests.

$\alpha$ -glucosidase inhibitors were approved as therapeutic antidiabetic drugs in the 1990s. Acarbose and voglibose are the most  $\alpha$ -glucosidase inhibitors used in the treatment of diabetes in combination with either diet or other anti-diabetic drugs (1).

$\alpha$ -glucosidase inhibitors showed a beneficial therapeutic effect against some diseases such as cancer, human immunodeficiency virus (HIV) and hepatitis B (2).

Most of the drugs given even today are directly or indirectly from natural sources (3). Medicinal plants provide a large

bank of chemical compounds, which we can explore for therapeutic agents after isolation and purification.

Several plants are found to produce many different  $\alpha$ -glucosidase inhibitors such as polyphenols, alkaloids, triterpene, flavonoids, xanthenes, phytosterol, etc. (4). Plants use  $\alpha$ -glucosidase inhibitors as a defense mechanism as a protection from insects. These inhibitors alter the digestive action of  $\alpha$ -glucosidase and proteinases in the gut of insects and inhibit their normal feeding behavior. Therefore,  $\alpha$ -glucosidase inhibitors have a potential role in plant protection (5).

*T. hirsuta* is a medicinal plant widely used in the northeastern Morocco to treat diabetes (6). Several studies have demonstrated the antihyperglycemic and the antidiabetic effect of this plant in various diabetic rat models (7–10).

On the other hand, *T. hirsuta* has shown antitumoral, anti-hepatocellular carcinoma and anti-human immunodeficiency virus (HIV) activities (11–13).

Concerning the action mechanism of the antihyperglycemic effect of *T. hirsuta*, our previous studies have demonstrated *in vitro* and *in vivo*, the potent effect of EaTh against  $\alpha$ -glucosidase (14).

To provide more candidates of  $\alpha$ -glucosidase inhibitors antidiabetic drugs choices, it is still necessary to search for new  $\alpha$ -glucosidase inhibitors. Thus, the aim of this study is to isolate and purify  $\alpha$ -glucosidase inhibitors from *T. hirsuta* by a simple and rapid approach. Generally, in the classical approach, isolation, purification and bioanalysis of  $\alpha$ -glucosidase inhibitors is a long chain process. Therefore, the present work aims to establish a new combination of thin layer chromatographic methods and enzymatic tests (TLC/EZ) to isolate and purify  $\alpha$ -glucosidase inhibitors without time and energy consuming.

## Materials and methods

### Chemicals and reagents

$\alpha$ -glucosidase (Sigma-Aldrich, USA) solution (33.6 IU/ml) and sucrose (Prolabo, groupe Rhone-Poulenc, EEC) solution (50 mM) were prepared with sodium phosphate buffer pH = 8.5. GOD-POD Autokit was purchased from BioSystems (Spain). Solvents were from Sigma-Aldrich (Steinheim, Germany).

### Plant material

*Thymelaea hirsuta* (Thymelaeaceae) was purchased from a traditional market in Oujda (Oriental Morocco) and was authenticated by a botanist in the Department of Biology (faculty of sciences, Oujda, Morocco) and a voucher specimen (HUMPOM137) was deposited at the plant section of the Herbarium University Mohamed Premier of Oujda, Morocco (HUMPO).

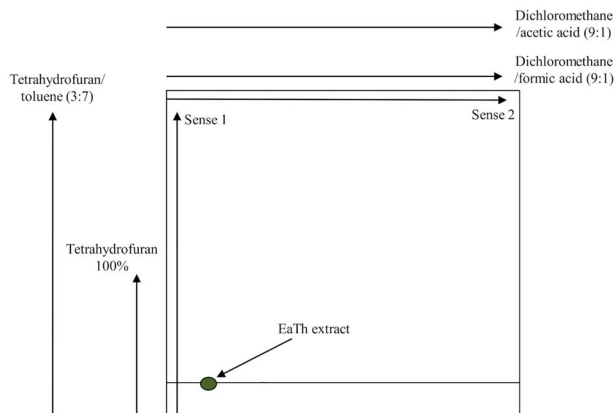
### Inhibition of $\alpha$ -glucosidase

#### Extraction, fractionation and isolation of actives compounds from EaTh

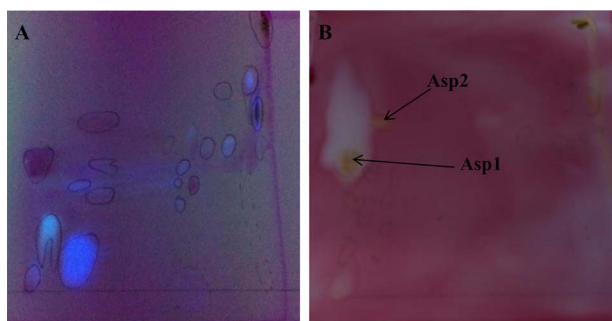
*T. hirsuta*'s aerial parts were first cleaned and washed with water and then dried in a circulating air oven at 40°C overnight. After drying, the plant was milled. The dried and ground *T. hirsuta* powder was subjected to a Soxhlet extraction to get EaTh as described in our previous study (14). The EaTh yield was  $0.95 \pm 0.03\%$ .

The obtained EaTh was stored in borosilicate glass vial at  $-20^\circ\text{C}$  until further use. To identify EaTh in analytic HPLC, 10 mg of sample was solubilized in 1 ml of methanol then filtered with a Whatman filter paper (0.2  $\mu\text{m}$ , PTFE).

Flash chromatography proceeded as follows; 300 mg of crude EaTh was solubilized in 20 ml of methanol and then 2 g of silica were added to AeTh. The mixture is evaporated on a dry ice evaporator to obtain a silica powder impregnated with AeTh. The powder is then filled in the cartridge connected to the silica gel chromatographic column (Silica 40  $\mu\text{m}$  [12 g], Revelris) and successively eluted with toluene (100–50% for 30 min) then with tetrahydrofuran (100% for 40 min). About, 129 fractions were collected, and each five fractions were spotted on a precoated silica gel plate (6  $\times$  20 cm) and eluted in toluene: tetrahydrofuran (4:6). After



**Figure 1.** Flowchart of the two-dimensional (2D-TLC) pattern of ethyl acetate extract of *T. hirsuta* (EaTh).



**Figure 2.** Two-dimensional TLC (2D-TLC) pattern of ethyl acetate extract of *T. hirsuta* (EaTh). A. Revelation at 254 and 366 nm. B. actives spots after  $\alpha$ -glucosidase inhibition test. Asp1: active spot 1 in two-dimensional TLC (2D-TLC), Asp2: active spot 2 in two-dimensional TLC (2D-TLC).

fractions migration,  $\alpha$ -glucosidase inhibition test was realized in the silica plate. Fractions with  $\alpha$ -glucosidase inhibitory activity in TLC pattern were pooled together as one active subfraction (Asf). The amount of Asf after evaporation on the dry ice evaporator was 45.8 mg. This quantity has been solubilized in 1.5 ml of methanol then filtered with a Whatman filter paper (0.2  $\mu\text{m}$ , PTFE) then it was subjected to HPLC–MS analysis.

In addition, a two-dimensional TLC (2D-TLC) was realized to isolate the maximum possible EaTh actives compounds. About, 10 mg of crude EaTh was dissolved in 1 ml of methanol and then it was filtered through Whatman filter paper (0.2  $\mu\text{m}$ , PTFE). The residue was spotted on a precoated silica gel plate (12  $\times$  12 cm). The elution was done in two senses as demonstrated in the scheme 1 (Figure 1). Sense 1: it was eluted in 100% tetrahydrofuran up to half of the plate then in tetrahydrofuran/toluene (3:7) up to the top of the plate. Sense 2: it was eluted in dichloromethane/formic acid (9:1) up to the top of the plate then in dichloromethane/acetic acid (9:1) up to the top of the plate. After the migration in these two senses,  $\alpha$ -glucosidase inhibition test was realized in 2D-TLC. Spots with  $\alpha$ -glucosidase inhibitory activity in 2D-TLC pattern were scratched and dissolved each in 0.5 ml methanol then filtered through Whatman filter paper (0.2  $\mu\text{m}$ , PTFE). The test showed two active spots (active spot 1: Asp1 and active spot 2: Asp2) (Figure 2B). Finally, the two active spots were subjected to HPLC–MS analysis.

### $\alpha$ -glucosidase inhibition method in TLC

The inhibitory activity of active spots in TLC and 2D-TLC was determined by GOD-POD method on TLC as a new easy and rapid method. The TLC and 2D-TLC plates were first sprayed with sucrose (50 mM) then with  $\alpha$ -glucosidase (33.6 IU/ml) in a sodium phosphate buffer (pH 8.5). The sprayed plates were incubated at room temperature for 30 min. Then, active spots detection was performed by spraying GOD-POD kit on the totality of the plates.

$\alpha$ -glucosidase enzyme degrades sucrose to fructose and glucose. In TLC and TLC-2D active spots, the  $\alpha$ -glucosidase is inhibited. So, sucrose cannot be degraded to fructose and glucose. The GOD-POD is a colorless kit but in contact with glucose gives a pinkish coloration. Therefore, according to this method, no active spots appeared pink and active spots appeared whit.

### Validation of the TLC $\alpha$ -glucosidase inhibition method

The TLC and 2D-TLC  $\alpha$ -glucosidase inhibition method was validated according to the ICH Q2(R1) and FDA guidelines considering different attributes such as linearity, accuracy and precision against  $\alpha$ -glucosidase. The linearity was checked on three days among the three individual TLC and 2D-TLC  $\alpha$ -glucosidase inhibition assay. The accuracy was measured by detecting the actual inhibitory effect of EaTh against  $\alpha$ -glucosidase measuring the diameter of the inhibition zone by repeating the assay in triplicates for 3 days. The precision of the method was expressed as a consistence degree between the results of analyses carried out repeatedly (15, 16).

All equipments and volumetric glasswares were evaluated and calibrated before analysis. The balance (Sartorius MSA-224S-000-DU Cubis Analytical Balance, Elk Grove, USA) was calibrated to minimal measures of 0.1 mg.

### HPLC-mass spectrophotometry characterization of EaTh, Asf and actives compounds in 2D-TLC

Stock solutions (10 mg/ml of EaTh and 30 mg/ml of Asf) were prepared in triplicate with methanol. Samples were injected in LC-MS in the same conditions as the validation studies.

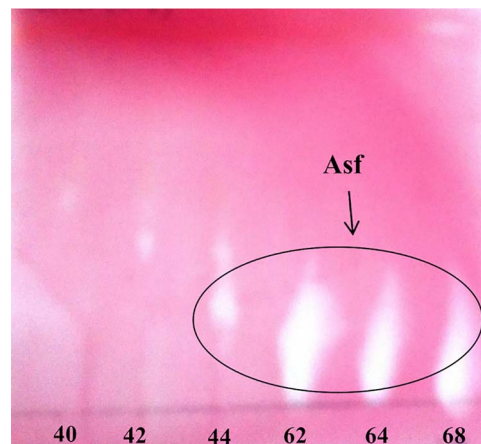
Identification of EaTh, Asf, Asp1 and Asp2 was performed by LC-MS using a Sunfier C18 column (2x100 mm, Agilent technologies, 1260 infinity, Waldbronn, Germany). The injected volume for all samples was 1  $\mu$ l. Elution was realized with a 20-min linear gradient of 40–100% acetonitrile in H<sub>2</sub>O at 0.3 ml/min with a positive ionization mode. The data acquisition and analysis were performed using “Mass & Hunter” software.

### Purification of active compounds

The Asf active compounds were purified by HPLC on a Sunfier C18 preparatory column (19  $\times$  150 mm, 5  $\mu$ m) eluted with a 20-min gradient of 20–40% acetonitrile in H<sub>2</sub>O at 17 ml/min. Fractions containing pure compounds were concentrated under a dry ice evaporator and lyophilized.

### Statistical analyses

All experiments were conducted in triplicates. All values were expressed as their mean and standard deviation.



**Figure 3.** Actives fractions, of ethyl acetate extract of *T. hirsuta* (EaTh), after  $\alpha$ -glucosidase inhibition test in TLC. Asf: active subfractions in TLC.

## Results

### $\alpha$ -glucosidase inhibition in TLC

The TLC-2D analysis of EaTh revealed the presence of 21 spots which absorb at 254 and 366 nm (Figure 2A). The  $\alpha$ -glucosidase inhibition test revealed a large white area of inhibition composed by two active spots: Asp1 and Asp2 (Figure 2B). On the other hand, the  $\alpha$ -glucosidase inhibition test on the TLC EaTh fractions showed that active fractions started from fraction 44 to fraction 68 (Figure 3). So, they collected as one active subfraction: Asf.

### Phytochemical analysis of active compounds

The analysis of EaTh, Asp1, Asp2 and Asf, chemical constituents were realized by LC/MS UV (254 nm). Peaks were characterized by the  $[M + H]^+$  molecular ion.

EaTh chromatogram showed five major peaks (Figure 4A). Peak 1: retention time (RT) = 9.03 min,  $[M + H]^+$ :  $m/z = 543.1$  corresponding to C<sub>30</sub>H<sub>22</sub>O<sub>10</sub> (Figure 5A). Peak 2: RT = 9.8 min,  $[M + H]^+$ :  $m/z = 545.1$  corresponding to C<sub>27</sub>H<sub>6</sub>N<sub>10</sub>O<sub>4</sub>. Peak 3: RT = 9.9 min,  $[M + H]^+$ :  $m/z = 659.1$  corresponding to C<sub>28</sub>H<sub>22</sub>N<sub>10</sub>O<sub>10</sub>. Peak 4: RT = 11.6 min,  $[M + H]^+$ :  $m/z = 271.2$ . Peak 5: RT = 12.4 min,  $[M + H]^+$ :  $m/z = 543.1$  corresponding to C<sub>30</sub>H<sub>22</sub>O<sub>10</sub> (Figure 5A').

Asp1 chromatogram showed one major peak (Figure 4B): peak 1: RT = 9.09 min,  $[M + H]^+$ :  $m/z = 543.1$  corresponding to C<sub>30</sub>H<sub>22</sub>O<sub>10</sub> (Figure 5B).

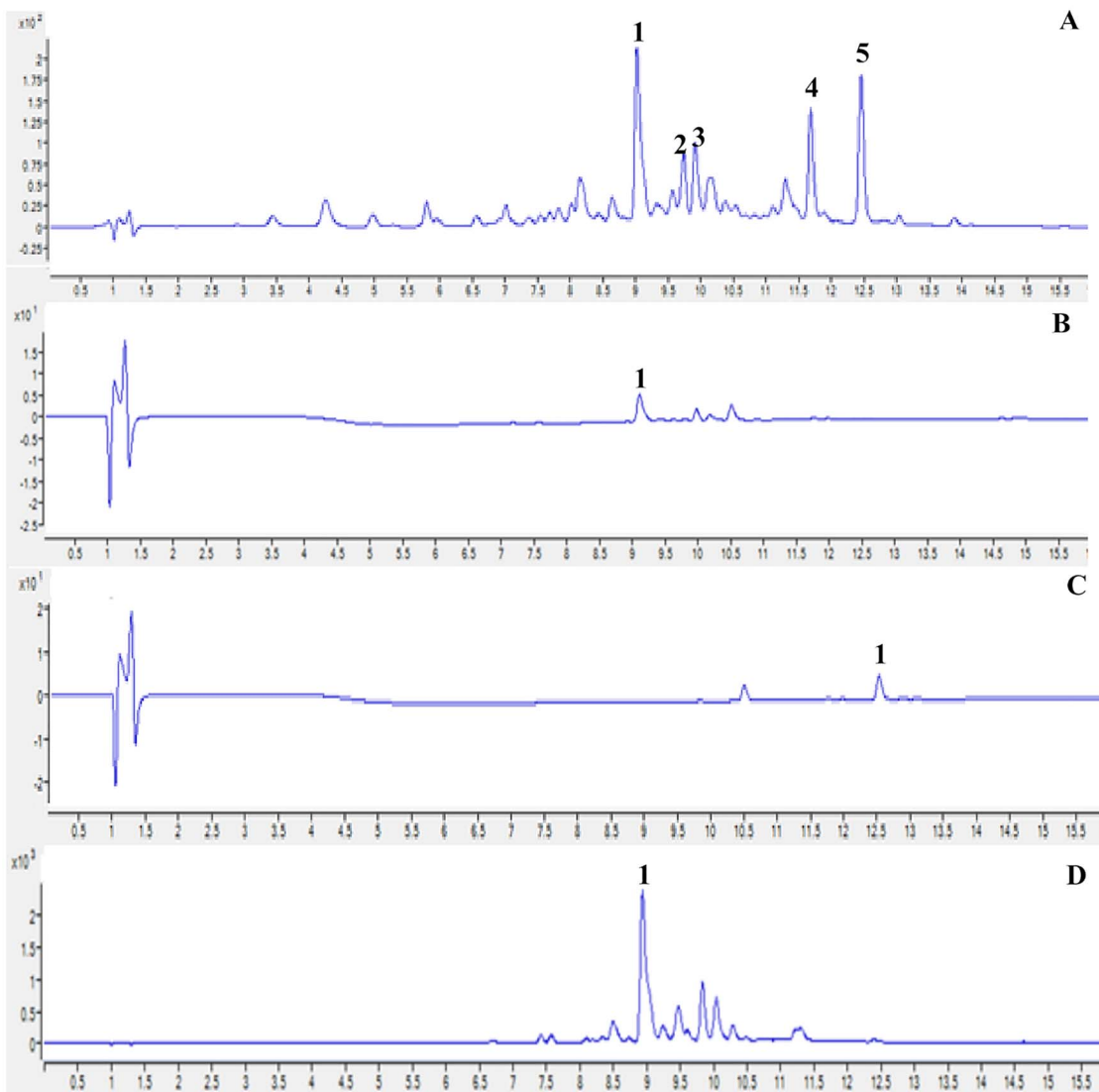
Asp2 chromatogram showed one major peak (Figure 4C): peak 2: RT = 12.48 min,  $[M + H]^+$ :  $m/z = 543.1$  corresponding to C<sub>30</sub>H<sub>22</sub>O<sub>10</sub> (Figure 5C).

Asf chromatogram showed one major peak (Figure 4D): peak 1: RT = 8.94 min,  $[M + H]^+$ :  $m/z = 543.1$  corresponding to C<sub>27</sub>H<sub>14</sub>N<sub>10</sub>O<sub>4</sub> (Figure 5D).

### Purification of active compounds

From Asf, six fractions were purified, which were revealed at 254 nm (Figure 6).

Fraction 1 = 7.2 mg, fraction 2 = unmeasurable, fraction 3 = 7.1 mg, fraction 4 = 8.2 mg, fraction 5 = 5.9 mg and fraction 6 = 13 mg. The identification of compounds of these fractions is in progress. So, for the moment we do not have any idea on compounds name.



**Figure 4.** HPLC chromatogram of ethyl acetate extract of *T. hirsuta* (EaTh) (A), Asp1 (B), Asp2 (C) and Asf (D) at UV 254 nm. Asp1: active spot 1 in two-dimensional TLC (2D-TLC), Asp2: active spot 2 in two-dimensional TLC (2D-TLC), Asf: active subfraction in TLC. A (1):  $C_{30}H_{22}O_{10}$ , A (2):  $C_{27}H_{16}N_{10}O_4$ , A (3):  $C_{28}H_{22}N_{10}O_{10}$ , A (4): non formula proposed, A (5):  $C_{30}H_{22}O_{10}$ . Formulas of compounds were provided according the "Mass & Hunter" software.

### Validation parameters of the TLC $\alpha$ -glucosidase inhibition method according to the ICH and FDA guidelines

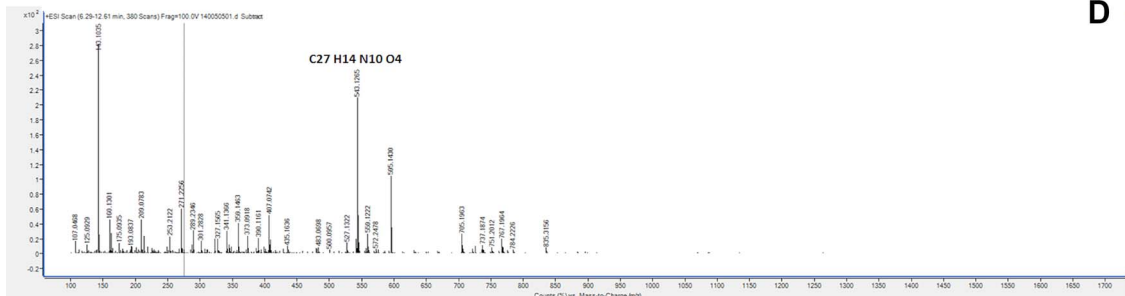
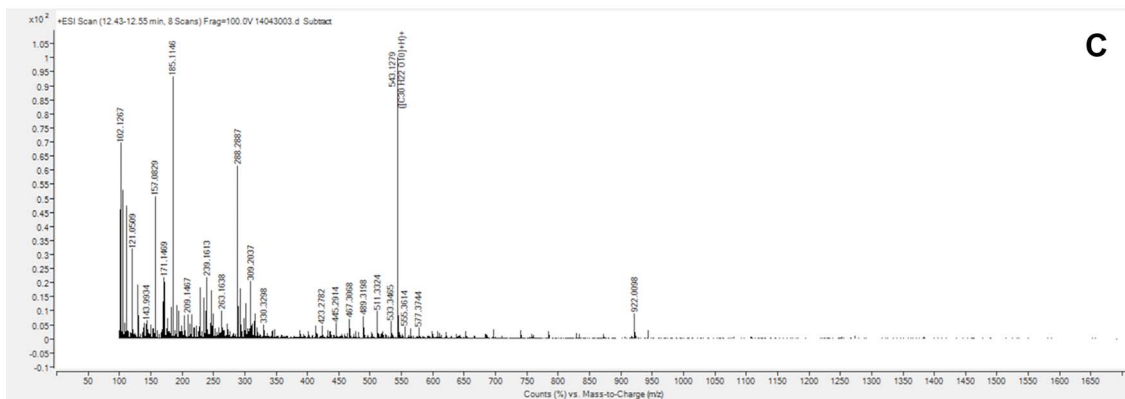
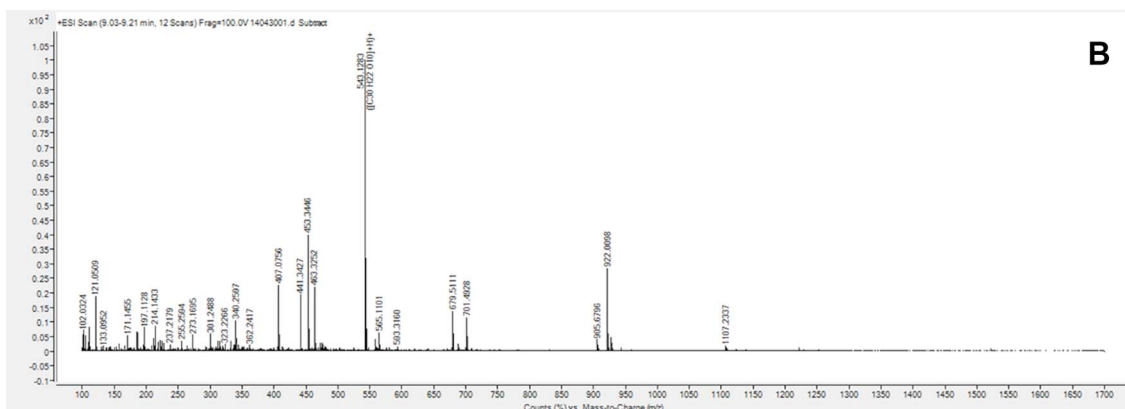
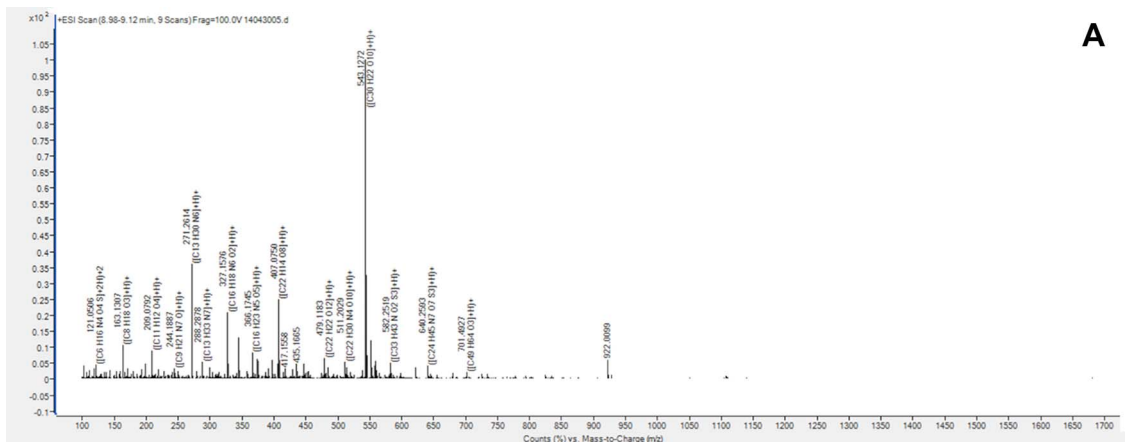
A linear regression curve was established to characterize the EaTh fractions/percent of inhibition zone relationship. Linearity was evaluated by plotting percent of inhibition zone against EaTh fractions (fractions from 40 to 68). The regression equation of calibration curve was  $y = 2.7467 \times x - 76.988$ ,  $R^2 = 0.7959$ . Where the  $y$  is the EaTh fraction,  $x$  is the percent of inhibition zone against  $\alpha$ -glucosidase and  $R^2$  is the regression coefficient (Table I). Linearity of the methods should be demonstrated by the slope of the linear calibration curve was statistically different from 0. The slope of the linear calibration curve was 2.7466, and it was statistically different from 0. All these results indicated that the method was linear.

Accuracy describes the closeness of mean results obtained to the true value of the concentration while precision describes the closeness of individual measures when the procedure is applied repeatedly (15). The diameter of the inhibition zone

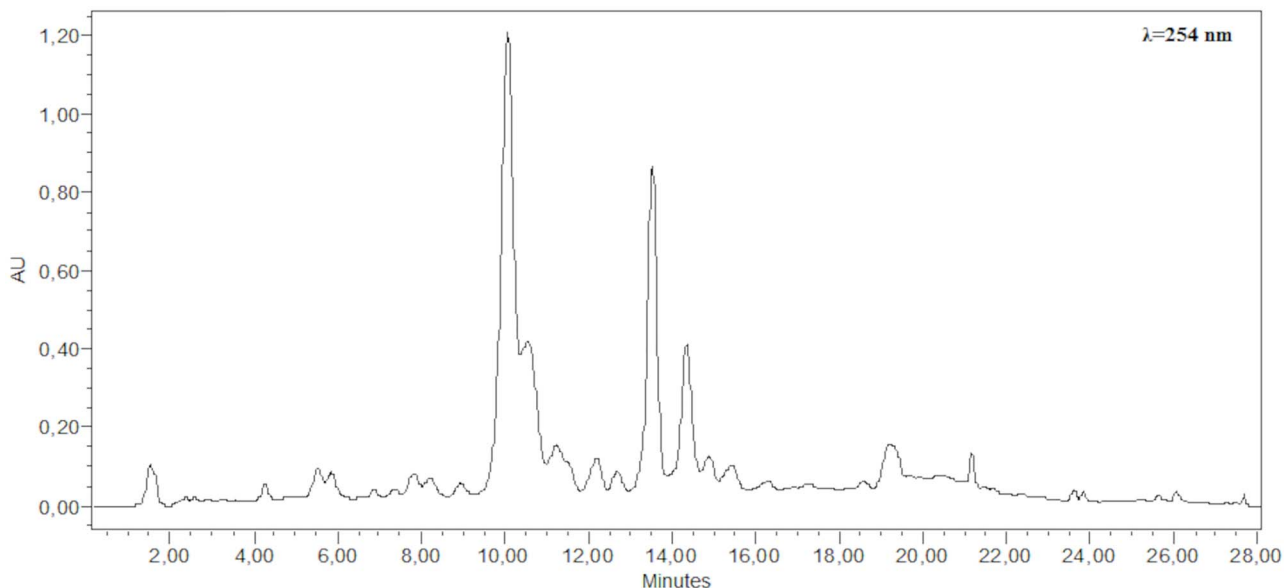
induced by the effect of EaTh against  $\alpha$ -glucosidase was measured three times within the same day and on three successive days. Accuracy was checked by percent of inhibition zone of EaTh against  $\alpha$ -glucosidase. The mean of the percent was found to be 98.79%. While precision was checked by percent relative standard deviation of the measured inhibition zone percent and found to be <2% for both intra-day and inter-day precision (Table I).

### Discussion

*T. hirsuta* is an important source to medicine due its complex mixture of polyphenols, flavonoids, tannins and alkaloids (17–22) with numerous pharmacologic activities (8, 18, 19, 22–24). Our research team has well studied the anti-diabetic effect of the plant and has also determined the mechanism of action of this effect (7, 9, 10, 14, 25). In fact, we have demonstrated that the antidiabetic effect of *T. hirsuta* involves the inhibition of  $\alpha$ -glucosidase. In this work, a new analytical



**Figure 5.** Mass spectrum (MS) of compounds, with masses ( $m/z$ ) and the proposed formulas, of major peak 1 of EaTh (A), major peak 5 of EaTh (A'), major peak 1 of Asp1 (B), major peak 1 of Asp2 (C) and of Asf (D), at UV 254 nm. EaTh: ethyl acetate extract of *T. hirsuta*, Asp1: active spot 1 in two-dimensional TLC (2D-TLC), Asp2: active spot 2 in two-dimensional TLC (2D-TLC), Asf: active subfraction in TLC.



**Figure 6.** Preparatory HPLC chromatogram of active subfractions in TLC (Asf) at 254 nm.

**Table I.** Validation parameters of EaTh fractions on the TLC  $\alpha$ -glucosidase inhibition method

Parameters	EaTh fractions
Linear equation	$y = 2.7467x - 76.988$
Regression coefficient ( $R^2$ )	0.7959
Accuracy (%)	98.79
Precision (%)	<2

method was proposed for rapid and reliable isolation and purification of  $\alpha$ -glucosidase inhibitors present in EaTh (*T. hirsuta*). EaTh was extracted by Soxhlet from *T. hirsuta*. In previous works, EaTh was showed a significant inhibition of  $\alpha$ -glucosidase *in vitro* and *in vivo* in normal and diabetic rats (14, 25). In this present approach, we succeeded in isolating and purifying  $\alpha$ -glucosidase inhibitors from EaTh using a new combination of thin layer chromatographic methods and enzymatic tests (TLC/EZ combination method). This in contracts with classical approach in which the analysis of bioactive compounds is based in the common phytochemical screening assays such as HPLC, HPLC/MS, TLC and NMR. Bioactive compounds are first extracted, isolated, and purified according to a long chain process (HPLC and TLC). Then, pure compounds are used for the determination of structure (NMR). Finally, the identified compounds are biologically tested *in vitro* and/or *in vivo*. Sometimes, the compounds are biologically active, but occasionally, they are not active. Therefore, the classical approach is time and energy consuming.

The TLC/EZ combination method was developed to identify  $\alpha$ -glucosidase inhibitors composing the EaTh. We showed before that EaTh is rich in  $\alpha$ -glucosidase inhibitors. To make easy and rapid their isolation and purification, the new method consists to isolate and purify these inhibitors from EaTh using a two-dimension TLC plate. In addition,  $\alpha$ -glucosidase inhibitors were characterized in the TLC plates using the GOD-POD method, as an alternative to the classical method *in vitro*. However, in the classical method, we scrape all spots from the TLC plate, then we tested, *in vitro*, all

these spots to determine  $\alpha$ -glucosidase inhibitors spots. So, the classical method is complicated and needs a long time. As an easy alternative, we decided to use the GOD-POD method in the TLC plate to determine  $\alpha$ -glucosidase inhibitors. This approach is generally helpful to easily isolate enzyme inhibitors present in plant extracts.

In the present study, we were based on TLC/EZ combination method developed here and on LC/MS analysis to isolate and purify  $\alpha$ -glucosidase inhibitors present in *T. hirsuta*. Generally,  $\alpha$ -glucosidase inhibitors analysis is an enzymatic assay that follows the basic principles as most enzymatic assays. Mostly, measurement of  $\alpha$ -glucosidase inhibitory activity is based on colorimetric methods. Sucrose is a substrate that is hydrolyzed specifically by  $\alpha$ -glucosidase into glucose (a pink red color product) that is usually quantified at 500 nm. Hence, measuring the amount of glucose produced from sucrose in the presence or absence of inhibitors is used to measure the inhibitory activity of plant compounds against  $\alpha$ -glucosidase (26). Applying the TLC/EZ combination method as a rapid isolation method, a good detection and isolation of  $\alpha$ -glucosidase inhibitors was showed in TLC. Mass spectrometer is considered as the most powerful detector for chromatographic methods. In addition, flam ionization detector is used due to its technical performances including adequate sensitivity, large linear response range and high signal to noise ratio for most needed analysis and because the equipment is more accessible to all analytical laboratories based on economic considerations (27). Furthermore, according to LC/MS, the data obtained in this work, showed that in EaTh as well as in Asp1, Asp2 and Asf the major peak component has a molecular ion  $[M+H]^+$  at  $m/z = 543$ , but they appear at different retention times. Therefore, we suggest that  $\alpha$ -glucosidase inhibitors present in *T. hirsuta* are isomers with a  $[M+H]^+$ :  $m/z = 543$ , which correspond to two different chemicals formulas:  $C_{30}H_{22}O_{10}$  and  $C_{27}H_{14}N_{10}O_4$  (LC/MS software).

Concerning the first formula, several molecules were proposed (by LC/MS software): Rhusflavanone, daphnodorin B, daphnogirin A, radicalisin, ivyflavanone, rugulosin, succedaneoflavanone, isochamaejasmin, chamaejasmin,

neochamaejasmin A, neo-chamaejasmin B, rubellin D, genkwanol A, yuccaol E, yuccaol D, yuccaol C, 2,3,2'',3''-tetrahydrochonaflavone, wikstroal A, rubroskyrin, larixinol, chamaechromone, 3,6''-binaringenin, 3-epi-larixinol, uredinorubellin I, abiesanol A, 2,3,2'',3''-tetrahydrochonaflavone, tetrahydroamentoflavone. Concerning the second formula, no molecule has been proposed. So, it can be a new molecule.

Consequently, it seems that many of these proposed molecules are flavonoids. Rhusflavanone, ivyflavanone and succedaneaflavanone are flavanones. 2,3,2'',3''-tetrahydrochonaflavone, 2,3,2'',3''-tetrahydrochonaflavone and tetrahydroamentoflavone belong to flavones. These two subclasses of flavonoids have proved to be effective  $\alpha$ -glucosidase inhibitors in many studies (28–30).

Flavonoids represent a large class of at least 6,000 phenolic compounds found in plants (31). They can reduce the risk of diabetes (32, 33) and they have been described as glucosidase inhibitors (34). The putative inhibitory effects of some flavonoids on  $\alpha$ -glucosidase activity, such as luteolin, amentoflavone, luteolin 7-O-glucoside, daidzein, etc., were confirmed in several studies (35, 36).

In a previous study of our scientific team, a polyphene rich fraction of *T. hirsuta* demonstrated a potent antidiabetic effect in diabetic rats (10). Therefore, we assume that the  $\alpha$ -glucosidase inhibitors present in *T. hirsuta* are probably one (or more) of the flavonoid molecules proposed in HPLC/MS analysis as it can be a new molecule.

Moreover, further experiments are in progress to identify the structure of the purified  $\alpha$ -glucosidase inhibitors by NMR and to test the effect of single compounds and mixture of them to test the possible synergetic effects against  $\alpha$ -glucosidase.

## Conclusion

This study mentions rapid and simple method to isolate  $\alpha$ -glucosidase inhibitors using a new combination of thin layer chromatographic methods and enzymatic tests (TLC/EZ combination method). Consequently, the present method may be applicable as an interesting alternative of the classical method in the study of  $\alpha$ -glucosidase inhibitors.

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## Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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