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IN VITRO ANTIDIABETIC ACTIVITY OF ESSENTIAL OIL OF TWO SPECIES OF *ARTEMISIA: ARTEMISIA HEBA-ALBA ASSO* AND *ARTEMISIA IFRANENSIS*

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Abstract

This work aims to use two medicinal plants of the genus *Artemisia* to inhibit two enzymes responsible for diabetes: α-amylase and α-glucosidase. Two essential oils EOAH and EOAI were obtained by hydrodistillation, respectively of *Artemisia heba-alba asso* and of *Artemisia ifranensis*. Its chemical compositions were made by gas chromatography and it identified 22 compounds for EOAH (73.86%) and 23 compounds for EOAI (73.09%). The major compounds of EOAH are: α-thujone (18.34%), camphor (17.66%), chrysanthenone (10.82%), eucalyptol (8.19%) and β-thujone (5.94%) and the major compounds of EOAI are: β- thujone (33.21%), α-bisabolone oxide B (11.25%), α-bisabolone oxide A (9.31%) and eremoligenol (4.99%). Antidiabetic activity tests were performed according to α-amylase inhibition assays and α-glucosidase inhibition assays using appropriate spectrophotometric readings. The results obtained show the moderate activity of two essential oils against acarbose, the drug for diabetics, so the two herbs present a new avenue of herbal medicine and medicinal chemistry to treat diabetes.

Keywords: *Antidiabetic activity, Essential oils, tests in vitro, α-Amylase, α-Glucosidase.*

Introduction

Diabetes is one of the oldest diseases known to man, the devastating effect of which is increasing day by day and gravely to an epidemic level which has harmful and fatal side effects. **[1].** It is a disease of disordered carbohydrate metabolism, which also affects proteins and fats which are caused by the total or relative insufficiency of the action of insulin. **[2].** Several mechanisms explain that the main risk factors responsible for the onset of diabetes mellitus are unbalanced diet, change in lifestyle, physical inactivity, obesity, and psychosocial stress that threatens individual's day after day **[3].**

Diabetes is experiencing a very significant expansion, according to the latest estimates, diabetes killed more than 300,000 Africans in 2017, and according to projections 41 million people will be diabetic by 2045 on the continent **[4].** Despite the use of hypoglycemic drugs as anti-diabetic drugs, diabetes and its complications constitute a major problem in the therapeutic management of diabetics and the success of the treatment would be of great interest, despite the progress of new therapeutic molecules **[5].** Modern drugs, including insulin and oral hypoglycemic agents (biguanides, sulfonylureas), their regular administration causes side effects **[6].** Recently, diabetologists have come to the evidence that a therapeutic supplement consisting of plant derivatives is necessary to optimize the treatment of diabetes **[7].**

The field of herbal medicine research has gained tremendous importance over the past decades and the demand for the use of natural products in the treatment of diabetes is increasing worldwide **[8-10].** The available literature shows that there are over 400 plant species showing antidiabetic activity **[11].** Although some of these plants have a great reputation in traditional medicine, many remain to be scientifically demonstrated. Scientific study of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and strategies **[12].**

This work is concerned with the evaluation of the antidiabetic activity of two essential oils of *Artemisia* plants: *Artemisia heba-alba asso* (EOAH) and *Artemisia ifranensis* (EOAI) by carrying out a series of *in vitro* tests.

Material and methods

Essential oils

Two plants *Artemisia heba-alba asso* (EOAH) and *Artemisia ifranensis* (EOAI) were collected in full flowering stage from their natural habitats in Midle Atlas (December 2020). Plant material was air dried, packed in paper bags, and kept in a dark and cool place until analysis. The plant material was milled in a blender for 3 min. The average particle size of milled herbs was 0.5 mm. Hydro-distillation (HD) was employed using a Clevenger type apparatus (3h) for the isolation of essential oil from two species. The essential oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until analysis **[13].**

Chemical analysis

The samples were analyzed by using a GC equipped with a flame ionization detector (GC-FID) to obtain the quantitative composition and by GC–MS for molecule identification. GC-FID analysis was performed using Gas Chromatographer (HP6890) with automatic pressure control, equipped with HP-5MS capillary column (30 m × 0.25 mm, ft 0.25 µm), FID detector set at 250 °C and fed with hydrogen/Air mixture, and a split-splitless injector set at 250 °C. The injection mode was split (1:50) and the injected volume was 1 µl. Nitrogen was used as carrier gas with a flow rate of 1.5 ml/min. The column temperature was programmed from 50 to 200 °C at heating rate of 5 °C/min. The apparatus was controlled by software computer system: ChemStation **[14].**

α-Amylase Inhibitory Assay

The test was realized pursuing protocol of Morais *et al.* (2020) **[15].** To prepare the substrate solution, a solution of 0.5 M Tris–HCl buffer at pH 6.9 was mixed with 0.01 M $CaCl_2$ (0.2 ml) and 2 mg of starch. The substrate solution was distributed into test tubes, boiled (for 5 min) and preincubated (at 37 °C for 5 min). Using DMSO, essential oils were dissolved and prepared at different concentrations of 0.25, 5 and 1 mg/ml. The essential oils solution (0.2 mL) at different concentrations was added to the test tube containing the substrate solution, and later on, the porcine pancreatic amylase (0,1 ml in Tris–HCl buffer (2 units/ ml)) was added. This

reaction was performed out at 37 °C for 10 min and then stopped by adding 0.5 ml of 50% acetic acid in each test tube. A centrifugation was carried on (3000 rpm for 5 min at 4 °C) and the supernatant absorbance was at 595 nm using a spectrophotometer. For this assay, acarbose was used as a positive control (α-amylase inhibitor). The experiments were repeated three times for each concentration. To calculate the α-amylase inhibitory activity, this formula was used:

$$\text{Percentage of inhibition } (\%) = \frac{ABS_{Control} - ABS_{Sample}}{ABS_{Control}} \times 100$$

After determining the α-amylase inhibitory activity of the different concentrations, the IC_{50} values were determined for the acarbose and essential oils (concentration required to inhibit 50% of α-amylase).

α-Glucosidase Inhibitory Assay

The test was realized pursuing the protocol of Wu *et al.* (2021) **[16]**. An amount of 50 µl of essential oils were prepared at various concentrations (0.25, 0.5 and 1 mg/ml) and incubated with the solution containing 10 µl of α-glucosidase (maltase) 1 U/ml and 125 µl of 0.1 M phosphate buffer (pH 6.8) for 20 min at 37 °C. To start the reaction, a solution of 20 µl of 1 M pNPG (4-Nitrophenyl-β-d- glucopyranoside) was added then incubated for half-hour. To terminate the reaction, 50 µl of 0.1 N Na_2CO_3 was added. The absorbance was measured at 405 nm using a spectrophotometer. For this assay, acarbose was used as a positive control (α-amylase inhibitor). The experiments were repeated three times for each concentration. The α- Glucosidase inhibitory activity was calculated by using the following formula:

$$\text{Percentage of inhibition } (\%) = \frac{ABS_{Control} - ABS_{Sample}}{ABS_{Control}} \times 100$$

After determining the α-glucosidase inhibitory activity of the different concentrations, the IC_{50} values were determined for the acarbose and essential oils (concentration required to inhibit 50% of α-glucosidase).

Statistical Analysis

The statistical analysis was done with XLSTAT for Windows. Values are expressed as the mean ± Standard Deviation. The IC_{50} is calculated using linear regression by plotting x-y and fitting the data with a straight line **[17]**.

Results and discussion

The chemical composition of essential oils of the genus *Artemisia* have been listed in several international and national works **[18-20]**. The composition of two essential oils that are the objective of this work identifies 22 compounds in EOAH with a percentage of 73.86% (Table 1) and 23 compounds in EOAI with a percentage of 73.09% (Table 2) with the abundance of oxygenated monoterpene molecules for all oils. The major compounds of EOAH are: α-Thujone (18.34%), Camphor (17.66%), Chrysanthenone (10.82%), Eucalyptol (8.19%) and β-Thujone (5.94%) and the major compounds of EOAI are: β- thujone (33.21%), α-bisabolone oxide B (11.25%), α-bisabolone oxide A (9.31%) and eremoligenol (4.99%). The two essential oils have dozens of compounds in common with each other especially β-thujone and other compounds even if with different concentrations to each other like α-thujone, or small amounts like α-pinene, β-pinene and 4-terpineol. Our results are in agreement with the composition reported by several studies, particularly those of Aljaiyash *et al.* (2018) **[21]** on EOAH oil, and Elazzouzi *et al.* (2018) **[22]** on EOAI oil.

The antidiabetic activity *in vitro* was carried out according to two tests α-Amylase Inhibitory Assay and α-Glucosidase Inhibitory Assay. Figures 1 and 2 give the results of two tests according to concentrations of essential oils 0.25, 0.5 and 1 mg/ml with the positive control Acarbose which an active principle known by its inhibitory activities of α-Amylase and α-Glucosidase. Analysis of the data shows that the two oils have the same results showing moderate activity compared to Acarbose. The IC_{50} of the inhibition of the tests carried out are mentioned in Table 3, hence the IC_{50} of α-amylase for essential oils are 1.946 mg/ml for EOAH and 1.796 mg/ml for EOAI and the IC_{50} of α -glucosidase for essential oils are 1.754 mg/ml for EOAH and 1.527 mg/ml for EOAI.

α-glucosidase inhibitors, particularly acarbose, are used to lower blood sugar in people with type 2 diabetes mellitus **[23]**. Acarbose inhibits the enzyme

in the intestinal epithelium responsible for the digestion of polysaccharides, and therefore decreases the absorption of glucose in the small intestine, thus limiting post-prandial glycemic excursions **[24]**. The hypoglycaemic effect is still significantly more modest than that of the above-mentioned medications, estimated to be around 0.5%. If it does not induce hypoglycemia, its use is limited by a frankly poor digestive tolerance caused by the influx of carbohydrates into the colon responsible for a significant gas production with the functional disorders that follow. It is contraindicated in chronic pathologies of the intestines and severe renal failure. For these reasons, it is more of an add-on anti-diabetic when glycated homoglobins are low **[25-26]**.

This information makes it possible to target herbal remedies to reduce the harmful effects of the drugs used in this treatment. The present study aimed to show the inhibitory action of two essential oils of *Artemisia heba-alba asso* and *Artemisia ifranensis* against the enzymes α-amylase and α-glucosidase, since the traditional use of *Artemisia* species in Africa includes the treatment of diabetes, and other uses are well documented in ethnopharmacological studies of Bora & Sharma (2011) **[27]**.

The hypothesis can target the presence of the α-thujone and β-thujone isomers which play the role of inhibiting two enzymes α-amylase and α-glucosidase. Keskes *et al.* (2018) **[28]** worked on several Fenugreek (*Trigonella foenum-graecum* L.) Seeds extracts, from where he found significant α-amylase inhibitory activity of the hexane extract which contains a 10.8% concentration of β- thujone. Belhadj *et al.* (2018) **[29]** targeted the essential oil of *Salvia officinalis* which has an interesting activity of inhibiting the enzyme α-amylase, and according to this work the essential oil is rich in α-Thujone and β-Thujone with concentrations 29.0% and 4.6% respectively. Khadka *et al.* (2021) **[30]** selected a series of herbal remedies that may improve the insulin signaling pathway through herbal medicine, thus, he cited compounds that inhibit the enzyme α-amylase at concentrations 10–100 µg/ml among them are α-Thujone and β-Thujone.

Conclusion

The results reported in this study show the effectiveness of the use of medicinal plants of the genus *Artemisia* in reducing the enzymes responsible for the disease of diabetes, particularly *Artemisia heba-alba asso* and of *Artemisia ifranensis* from *in vitro* tests of the inhibition of two enzymes α-amylase and α-glucosidase. The two essential oils of these two plants contain the major chemotypes cited in the literature: α-thujone and β-thujone, which are proposed as effective inhibitors of the enzymes responsible for diabetes.

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Table 1. Chemical constituents of EOAH.

Peak	Component	(%)
1	α-Pinene	0.12
2	Camphene	3.42
3	β-Pinene	0.37
4	Sabinene	0.28
5	β-Myrcene	0.41
6	α-Terpinene	0.10
7	p-Cymene	0.27
8	Eucalyptol	**8.19**
9	γ-Terpinene	0.35
10	Filifolone	1.46
11	α-Thujone	**18.34**
12	β-Thujone	**5.94**
13	Chrysanthenone	**10.82**
14	Camphor	**17.66**
15	Pinocarvone	2.32
16	4-Terpineol	0.78
17	Carvone	0.78
18	α-Copaene	0.12
19	Bornyl acetate	0.11
20	Germacrene D	0.55
21	Spathulenol	1.32
22	Ledol	0.15
Total identified		73.86

Table 2. Chemical constituents of EOAI.

Peak	Component	(%)
1	α-Pinene	0.10
2	β-Pinene	0.56
3	α-Terpinene	0.10
4	p-Cymene	0.64
5	1,8-cineol	1.46
6	cis-Sabinene hydrate	0.64
7	Linalool oxide	0.14
8	α-Thujone	1.79
9	β-Thujone	**33.21**
10	α-Fenchol	0.54
11	Terpinen-4-ol	1.48
12	α-Isomethyl Ionone	0.42
13	γ-Isomethyl Ionone	0.74
14	Eremoligenol	**4.99**
15	α-Cadinol	2.27
16	Patchouli alcohol	0.11
17	α-Bisabolone oxide B	**11.25**
18	Valeranone	0.45
19	α-Bisabolone oxide A	**9.31**
20	α-Bisabolol oxide A	2.55
21	Cedryl acetate	0.10
22	8-Cedren-13-ol, acetate	0.10
23	α-Chenopodiol	0.14
Total identified		73.09

Table 3. IC_{50} (mg/ml) values of α-amylase and α-glucosidase inhibition assays.

Test	Acarbose	EOAH	EOAI
α-amylase inhibition assay	0.899	1.946	1.796
α-glucosidase inhibition assay	0.694	1.754	1.527

Figure 1. Results of the inhibitory effect of α-amylase of the two essential oils and of Acarbose.

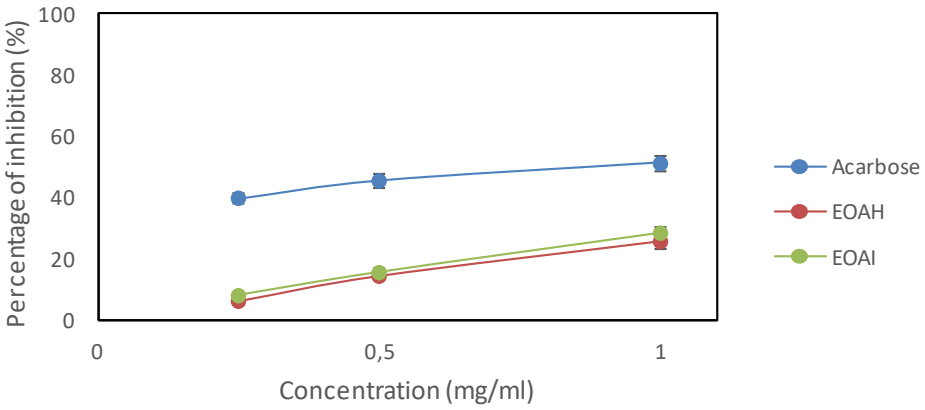

Figure 2. Results of the inhibitory effect of α- glucosidase of the two essential oils and of Acarbose.