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Research Article

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In vitro α-Amylase Inhibitory Activity of the Essential Oils of *Pinus nigra* ARN.: A Preliminary Assay

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Abstract

Such as several plants, Pines are known to have anti-diabetic potential. The current study evaluated the anti-diabetic potential of *Pinus nigra* essential oils, obtained from nine *Pinus nigra* provenances planted in Northwestern Tunisia, via in vitro inhibition of α-amylase. The α-amylase inhibitory potentials were determined using radial diffusion method. Our results showed a significant difference between provenances. The α-amylase inhibitory potential ranged from 10% to 26.6%. The essential oils of Crimée provenance from *Pinus nigra pallasiana* subsp. displayed the most effective inhibition of α amylase. While, essential oils of Bois frerot provenance from *Pinus nigra laricio* subsp. showed the lowest inhibitory potential (10%). The observed inhibitions of α-amylase suggest that the essential oils of *P. nigra* of some provenances may be useful in the management of diabetes mellitus.

Keywords

Pinus nigra, Subspecies, Essential oils, Provenances, α-Amylase inhibitory activity

Introduction

Diabetes is a metabolic complex disorder which can be the cause of severe health problems such as hypo or hyper glycemic and coma. It is, sometimes, accompanied by different other complications which can be further difficult to treat than diabetes. Actually, diabetic mellitus is treated by hypoglycemic drugs presenting, almost several side effects. Regarding the large increase in the number of diabetics in the entire world, many researchers have treated the pharmacological effect of several plants [1, 2]. Pines are considered, according to the literature, among the plants that have shown significant anti-diabetic power. Capetti et al. [3] determined the α-amylase inhibitory potential of essential oils from needles of *Pinus mugo* and *Pinus sylvestris*. In the same context Yang et al. 4] investigated the α-amylase inhibitory activity of essential oils extracted from the needle of *Pinus tabulaeformis*. To the best of our knowledge, no study was conducted on *Pinus nigra* essential oils. The present investigation evaluated, for the first time, the anti-diabetic potential of essential oils, obtained from nine *Pinus nigra* provenances growing in comparative plantation in Northwestern Tunisia, via *in vitro* inhibition of α-amylase.

Materials and Methods

Plant material

Needles of nine samples of *Pinus nigra* (Table 1) were collected from Souiniet arboretum located in West-Northern Tunisia (8 ° 48 'E, 35 ° 54' N,

Table 1: Geographic origin of the nin provenances of *Pinus nigra*.

Sub-species	Provenances	Country	Code
Pinus nigra laricio var. calabrica	Cosenza	Italy	P 1
Pinus nigra laricio var. corsicana	Bois frerot (Ardennes)	France	P 2
Pinus nigra nigra var. austriaca	Puget-Théniers	France	P 3
Pinus nigra nigra var. nigricans	Kustendil	Bulgaria	P 4
Pinus nigra laricio var. calabrica	Cantanzaro	Italy	P 5
Pinus nigra laricio var. corsicana	les Barres (leint)	France	P 6
Pinus nigra pallasiana	Crimée	Russia	P 7
Pinus nigra laricio var. calabrica	Tavola	Italy	P 8
Pinus nigra salzmanni	Olette (Pyr-Orient)	France	P 9

492 m).The plant was identified by Pr. Abdelhamid Khaldi, a specialist in plant identification. The nine samples correspond to provenances from different Mediterranean regions, which have been planted in the North West of Tunisia in 1966.

Oil extraction

Needles were air-dried and then used to extract essential oil by hydrodistillation. A Clevenger apparatus was used according to the method described by the British Pharmacopoeia [5]. The essential oils were than stored at 4 °C until analysis.

Oil yield

Oil yield was calculated as the percentage (%) of grams of extracted essential oil per gram of used dry needles.

α -amylase inhibitory test

The α-amylase inhibitory potentials were determined using radial diffusion method. 0.1786 g of α-amylase from *Aspergillus oryzae* was dissolved in 1 mL of 20 mM phosphate buffer and 6 mM of NaCl. The pH value was adjusted to 6.9. To each plate, containing 25 mL of agar-starch, 20 µL of α-amylase solution mixed with 10 µL of essential oil were inoculated. Clear diffusion zones were formed after incubation at 20 °C for 4 hours. The percentage of inhibition was calculated according to the following formula:

$$I = \frac{DE - / DC}{DC} x\ 100$$

Where: DE: Diameter of extract and DC: Diameter of Control

α-amylase inhibitor of wheat seeds (*Triticum aestivum*) was used as the positive control. Results are expressed as the mean of three replications.

Statistical analysis

The General Linear Models procedure (GLM) of the SAS (9.0) program was used for the statistical analysis.

Results

Significant variability was determined between the nine provenance ($p<0.0001$). The most important yield was recorded by needles of Puget Théniers provenance (0.68% ± 0.05), however the lowest one was found by les Barres (leint) of *laricio* subspecies (0.26% ± 0.04) (Table 2).

Results of α-amylase inhibitory activity showed that the highest effect was recorded for oils from Crimée (Russia) of *pallasiana* subspecies (26.6%) followed by oils from Cosenza (Italy) and from Olette (France) of both *laricio* and *salzmanni* subspecies, respectively (25%) (Figure 1). This inhibition is slightly lower than that of the positive control (35%).

The lowest inhibitory effect was reached by oils from Bois frerot (France) of *laricio* subspecies (10%).

Discussion

The present study was conducted to determine the potential of essential oils from nine provenances of *P. nigra* subspecies to inhibit α-amylase enzyme *in vitro*. Significant inhibition effect was demonstrated between the studied oils extracted from needles from different provenances. This variation can be related to the differences in oil composition demonstrated by Fkiri et al. [6].

The potential of essential oils to inhibit key carbohydrate hydrolyzing enzyme has long been studied. Several studies reported the efficiency of these natural products as antidiabetic compounds [7-9]. The anti-diabetic potential of some *Pinus* species were investigated. Capetti et al. [3] studied the α-amylase inhibitory potential of essential oils from needles of *Pinus mugo* and *Pinus sylvestris*. According to their findings,

Table 2: The yield of *Pinus nigra* essential oil.

Provenance	Oil yield (%)
P 1	0.37^{f} ± 0.02
P 2	0.41^{e} ± 0.04
P 3	0.68^{a} ± 0.02
P 4	0.38^{f} ± 0.02
P 5	0.51^{c} ± 0.05
P 6	0.26^{i} ± 0.04
P 7	0.41^{e} ± 0.01
P 8	0.66^{b} ± 0.02
P 9	0.40^{e} ± 0.01

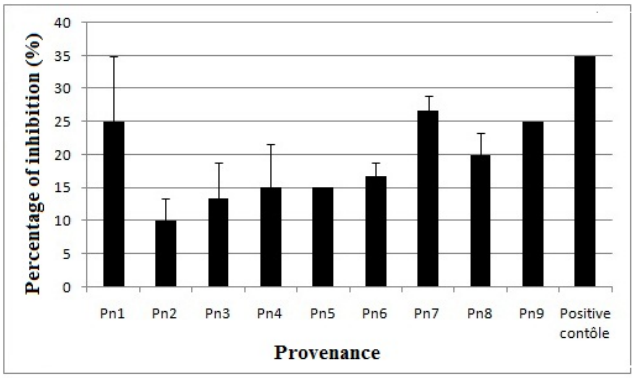

Figure 1: Percentage of α-amylase inhibition by essential oils of *Pinus nigra.*

no activity was observed for the two plants. Yang et al. [4] determined the α-amylase inhibitory activity of essential oils extracted from the needle of *Pinus tabulaeformis.* An important activity was recorded. It was attributed to terpenoids which are the main types of compounds in the essential oils. The effect of these compounds was highlighted by several studies [10, 11]. Terpenoids have been shown to be the main compounds of *P. nigra* essential oils [12]. This could explain the α-amylase inhibitory effect demonstrated in our study. To the best of our knowledge, no study addressed the antidiabetic potential of essential oils from *Pinus nigra*.

Conclusions

This study deals with the preliminary screening of essential oils from needles of *P. nigra* coming from different provenances and subspecies, in order to evaluate their potential hypoglycemic activity.

The observed inhibitions of α-amylase suggest that the essential oils of *P. nigra* of some provenances may be useful in the management of diabetes mellitus.

More experiments are required to support and deepen these findings. The actives molecules have to be identified and isolated.

Conflict of Interest

Authors declare no conflict of interest.

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