

Proceeding Paper

# Myco-Chemical Constituents and Anti-Inflammatory Activity of *Terfezia claveryi* Chatin from Algeria <sup>†</sup>

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**Abstract:** Mushrooms are receiving particular attention as a new source of valuable biotherapeutics. The aim of the current study is the valorization of *Terfezia claveryi* Chatin from Algeria. The myco-chemical constituents, polyphenol, flavonoid and condensed tannin composition and the in vitro anti-inflammatory activity was examined using the heat denaturation protein inhibition method. Myco-chemical tests presented a very interesting richness in terms of secondary metabolites, the polyphenol, flavonoid and condensed tannin contents of the hydro-methanol extract were respectively:  $82.27 \pm 1.44$  µg GAE/mg,  $14.94 \pm 0.98$  µg CE/mg,  $27.50 \pm 2.50$  µg CE/mg. The extract at the 1.5 mg/mL level showed an inhibition of denaturation  $83.53 \pm 1.57\%$  compared for that the diclofenac sodium  $98.43 \pm 0.52\%$ . This research revealed its interesting anti-inflammatory properties, which confirms its value in traditional use.

**Keywords:** *Terfezia claveryi*; myco-chemical constituents; anti-inflammatory activity

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## 1. Introduction

Truffles are a naturally-occurring foodstuff, renowned for its beneficial effects on health as an excellent bio-source. The desert truffles are hypogeous fruiting bodies, symbiotic fungi of ascomycetes found in arid and semi-arid regions, widely used in traditional medicine for their nutritional richness and therapeutic effects [1–3]. Due to their content of protein, vitamins, minerals and dietary fiber, truffles have a particularly high nutritive value [4]. In recent years, truffle secondary metabolites have received considerable attention for their anti-inflammatory, antioxidant, antimicrobial and antimutagenic effects [5]. The truffles harvest usually varies from season to season, according to the abundance of rainfall [6]. An interesting observation is that that the chemical composition of truffles of the same species from different regions is not always the same, it is Most probably determined by a wide range of environmental factors, such as the amount and timing of rainfall, types of soil and climatic variations [7]. In spite of its nutritional advantages, little attention has been paid to the biological activities and phytochemical substances of *T. claveryi* [8].

The present study was carried out to evaluate the in vitro anti-inflammatory activity and myco-chemical constituents of the desert truffle *T. claveryi*.

## 2. Materials and Methods

### 2.1. Extract Preparation

The desert truffles were harvested during December 2020, around Tlemcen region (El Aricha), Algeria. They were air-dried at room temperature. The dried parts were then ground into powder. The powder was extracted with a methanol/water mixture (70:30 *v/v*) by maceration at room temperature for 48 h. The mixture was then filtered and concentrated using a rotary evaporator [9,10].

### 2.2. Mycochemical Analysis

The extract obtained from *T. claveryi* was subjected to various qualitative myco-chemical tests to identify the different families of secondary metabolites present by coloration and precipitation reactions. These tests were carried out according to the techniques described by [11,12].

### 2.3. The Polyphenol Content

The polyphenol content of the extract was determined using the Folin-Ciocalteu method described by [13,14]. 0.1 mL extract (1 mg/mL) was mixed with 2 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution (2% *w/v*). After 5 min incubation, 0.1 mL of Folin-Ciocalteu reagent (1N) was added. The mixture was incubated for 30 min at room temperature in the dark. Absorbance readings were taken against a blank using a spectrophotometer at 725 nm. A calibration curve was performed under the same experimental conditions, using gallic acid as a standard. Total polyphenol levels were calculated using the linear regression equation. Results were expressed in equivalent micrograms of gallic acid per milligram of dry extract ( $\mu\text{g EAG/mg}$ ).

### 2.4. The Flavonoids Content

Flavonoids were quantified in the extract using the aluminum trichloride method described by [15]. 250  $\mu\text{L}$  of the sample was mixed with 75  $\mu\text{L}$  of a 15% (*w/v*)  $\text{NaNO}_2$  solution and 1 mL of distilled water, after a 6 min rest, 75  $\mu\text{L}$  of a 10% (*w/v*)  $\text{AlCl}_3$  solution was added, following a 5 min incubation, 1 mL of 4% (*w/v*)  $\text{NaOH}$  was introduced. The mixture was adjusted to 2.5 mL with distilled water, and the absorbance was read at 510 nm against a blank after 15 min. Total flavonoid content was calculated using catechin as standard ( $\mu\text{g CE/mg}$ ).

### 2.5. The Total Tannin

The total tannin content of the extract was estimated using the method [16]. 1.5 mL of 4% (*v/w*) vanillin/methanol solution and 750  $\mu\text{L}$  of concentrated  $\text{HCl}$  were added to 50  $\mu\text{L}$  of the extract (1 mg/mL). Measurement was taken at 550 nm after 20 min of reaction at room temperature. The condensed tannin content was determined from the linear regression equation of the catechin calibration curve. Results are expressed as microgram catechin equivalent per milligram extract ( $\mu\text{g CE/mg}$ ).

### 2.6. Anti-Inflammatory Activity

Anti-inflammatory activity *in vitro* was assessed by the procedure of [17] with slight modifications. The basic principle of this method is the inhibition of heat (72 °C) induced BSA denaturation by extracts. 1 mL of 0.2% (*w/v*) BSA solution (prepared in Tris-HCl, pH: 6.3) was mixed with 1 mL of different concentrations of extract preparations (or standard). After incubation for 15 min at 37 °C, denaturation was induced by heating in a water bath for 5 min at 72 °C, followed by cooling. The control consisted of 1 mL distilled water with 1 mL 0.2% BSA, under the same operating conditions. Absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 660 nm.

Denaturation inhibition percentage (%) =  $[(Ab \text{ control} \times Ab \text{ sample})/Ab \text{ control}] \times 100$ .

### 3. Results and Discussion

#### 3.1. Mycochemical Screening

The mycochemical tests consist in detecting the various existing chemical families by means of qualitative characterization reactions. The results of mycochemical screening are shown in the Table 1.

**Table 1.** Myco-chemical screening results obtained from the hydro-methanolic extract of *T. claveryi*.

Mycochemical Tests *	Extract of <i>T. claveryi</i>
Alkaloids	+
Flavonoids	+
Tannins	+
Anthraquinones	-
Coumarins	+
Terpenoids	+
Reducing sugars	+

+: positive; -: negative.

The measurement of total polyphenols, flavonoids and condensed tannins revealed the presence of these compounds in the hydro-methanolic extract in varying quantities. Total phenolic components are the main bioactive constituents found in the extract, followed by condensed tannins and flavonoids. The results obtained are shown in Table 2. A large number of scientific studies demonstrate that the consumption of a polyphenol-rich diet helps reduce the risk of chronic diseases, with health benefits for the human body [18,19].

**Table 2.** Total phenolic content, total flavonoid content and total condensed tannins of the hydro-methanolic extract of *T. claveryi*.

	Extract of <i>T. claveryi</i>
Total polyphenols ( $\mu\text{g GAE/mg}$ )	$82.27 \pm 1.44$
Flavonoids ( $\mu\text{g CE/mg}$ )	$14.94 \pm 0.98$
Condensed tannins ( $\mu\text{g CE/mg}$ )	$27.50 \pm 2.50$

Values are expressed as mean  $\pm$  standard deviation; GAE: gallic acid equivalent; CE: Catechin equivalent positive; -, negative.

#### 3.2. Anti-Inflammatory Activity

Inflammation plays a crucial role in the elimination of pathogens, serving as a protective immune response. However, an uncontrolled or chronic inflammatory response can contribute to the genesis of many chronic inflammatory diseases [20].

The results of the in vitro anti-inflammatory activity of the extract are shown in the Table 3. It can be seen that the percentage inhibition of BSA denaturation (0.2%) is proportional to the different concentrations of the extract, where the strongest protection rate  $83.53 \pm 1.57\%$  is registered at a concentration of 1.5 (mg/mL). However, these results are nearly proximate to those obtained for the diclofenac sodium (a non-steroidal anti-inflammatory drug) applied as a standard, which almost completely prevents BSA denaturation with a percentage of  $98.43 \pm 0.52\%$  at the same concentration. This potency may be attributable to the presence of polyphenols. These components exert considerable anti-inflammatory properties, and can be regarded as contributing to their prevention [21–23].

**Table 3.** Effect of extracts on inhibition of BSA denaturation (in percentage).

	0.75 (mg/mL)	1.5 (mg/mL)
Extract of <i>T. claveryi</i>	69.41 ± 1.57	83.53 ± 1.57
Diclofenac sodium	92.16 ± 1.31	98.43 ± 0.52

#### 4. Conclusions

The current study underlines the importance of bioactive compounds, which could have pharmacological properties and therapeutic potency, thus demonstrating the potential anti-inflammatory properties of the hydro-methanolic extract of *T. claveryi*. More research is required, to identify the main bio-compounds responsible for this remarkable potency, and their mechanism of effect.

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