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TOTAL POLYPHENOL CONTENTS, RADICAL SCAVENGING AND CYCLIC VOLTAMMETRY OF ALGERIAN PROPOLIS

ABDELKERIM REBIAI*¹, TOUHAMI LANEZ² AND MOHAMED LAKHDER BELFAR¹

¹University of Ouargla, VPRS Laboratory, B.P. 511, 30000, Ouargla, ²University of El Oued, VTRS Laboratory, B.P.789, 39000, El Oued, Algeria.

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ABSTRACT

Objective: In this study, we will determine the antioxidant properties of methanolic extract of propolis from Ghardaia and Khanchla provinces of Algeria and will correlate the values with total levels of polyphenolic compounds.

Methods: The total polyphenol contents of methanolic extract of propolis were measured by using Folin-Ciocalteu spectrophotometric method. Thereafter, the antioxidant properties of these polyphenols were determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. All polyphenols extracted were tested using cyclic voltammetry (CV) in aqueous media. The CV was realised to compare the results from spectroscopic method and to electrochemically characterise the propolis polyphenols.

Results: The total polyphenolic content in methanolic extract of propolis from Ghardaia and Khanchla was 493.49 and 1423.32 mg gallic acid equivalent/100g of extract, respectively. The IC₅₀ values for scavenging DPPH radical for Ghardaia and Khanchla propolis were 0.03917 mg/mL, 0.01211mg/mL respectively. Antioxidant activity measured by cyclic voltammetry method indicated that methanolic extract of Khanchla propolis had AEAC of 15,61 mg/g.

Conclusion: Propolis samples had strong antioxidant activities, and the highest activities were found in Khanchla propolis. Also, among three assays employed in this study (DPPH, RP, CV), the cyclic voltammetry method was recommended as it represented a relatively clean chemical system.

Keywords: Propolis, Polyphenol, Flavonoid, Cyclic voltammetry, Antioxidant.

INTRODUCTION

Bee propolis or bee glue is a very sticky valuable resinous mixture produced by honeybees from trees bubs and various plants sources around the hive [1], it is masticated by the bees, salivary enzymes and beeswax added, then used as a construction material in bee hives for filling cracks and repairing combs thereby insulating and reinforcing the hives [2], also protecting the hive and its nutritious contents from attack by micro-organisms [2,3]. Due to biological and pharmacological activities, propolis has been extensively used in folk medicine since ancient times and is now known to be a natural medicine with antibacterial, antifungal, antitumoral, antioxidative, immunomodulatory and other beneficial activities [4]. Now, propolis is presently used in health food and various pharmaceutical [4,5] and cosmetic products such as mouthwash preparations, face creams, lotions and tablets [5]. Propolis contains a diversity of compounds of plant origin. It is, basically, composed of 55% vegetable resins and balsam, 30% bee wax, 10% essential oils and 5% of pollen [2].

Propolis, in more of their high content in vitamins and minerals [6], contain important content of polyphenols [7]. These compounds are known today for their antioxidant capacities.

Although the considerable importance of antioxidants, there is not a unique method or protocol for the determination of antioxidant capacity (ORAC, FRAP, TEAC). Therefore, the results obtained from all these methods are not constantly compatible and the materials used for these analyses are costly (AAPH, ABTS, DPPH). For those reasons, we applied a new technique to determine antioxidant capacities using less complicated methods, compared to chromatographic and spectroscopic techniques which use complicated apparatus.

A highly attractive, convenient and especially sensitive voltammetry approach for the study of antioxidant properties [8], antioxidants are substances, which interrupt radical-chain oxidation in organic and inorganic molecules. Our method consists in using electrochemical techniques, cyclic voltammetry (CV), for the determination of propolis antioxidant capacities.

Voltammetry methods appear as simple methods giving good estimations of the global amount of polyphenols in vegetables and plants [7,8,9]. Furthermore, these techniques have been capable to

give the global amount of different types of polyphenols in the same time and to characterise new compounds containing polyphenols which could play important role in food [8].

Thus, in the present paper, the first part is focused on spectroscopic techniques used to study propolis extracts, in purpose to determine their total polyphenol contents (TPC). It should be noted that in the spectroscopic section we used Folin-Ciocalteu method to determine their polyphenols consistence. In the second part of this study we were interested to study their antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl DPPH as a scavenging radical reagent. Also, and for the main reason in the third part, these extract's were tested electrochemically, using cyclic voltammetry, to determine their electrochemically response. This technique can provide us the composition of each extracts, qualitatively, and can give us the global amount of polyphenols in each extract.

MATERIALS AND METHODS

Chemical

Methanol (99%), Folin Ciocalteu reagent, trichloroacetic acid (99%), potassium chloride (99.8%) was all purchased from biochem chemopharma Co (Canada).

1,1-Diphenyl-2-picryl hydrazyl (DPPH) (99%), potassium ferricyanide (99%), ascorbic acid (99.7%), gallic acid (99%) ferric chloride (99%), sodium carbonate (99%), AlCl₃ (99%), rutin (99%) were all purchased from Merck Co. Orthophosphoric acid (85%) was purchased from Riedel-de Haen Co, all other reagents used were of analytical grade.

Propolis

Two Crude propolis samples were brought from hives of honeybees located in Ghardaia (south of Algeria) and Khenchela (east of Algeria) in May-April, 2007. The samples, once received, were stored at 4°C in airtight /dark plastic containers until analysis.

Methods

Instrument

UV-Visible spectrophotometer (PRIM Advanced SCHOTT Instruments GmbH), centrifuge Machine (SLW centryge, Ultra-8TL), PGP301