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Ultraviolet's absorption spectrum and antioxidant activity of ethanolic extract from the bark of medicinal herb *Terminalia catappa*

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Abstract

Polyphenols are the most abundant antioxidants in the diet. Catechin, a secondary plant metabolite, is a natural phenol with powerful antioxidant activity. Nowadays there is considerable interest in finding out about antioxidants that are consumed in the habitual diet, and which ones are affordable and have easy access. One method was chosen to determine the UV absorption spectrum (200-400 nm): a single beam spectrophotometer with quartzo cuvettes and optical path of one centimeter. This work aimed to study and compare the *in vitro* antioxidant activity of the ethanolic extract from the stem bark of *Terminalia catappa* (EETCc), catechin and butylated hidroxy toluene (BHT). In general, it was demonstrated that EETCb antioxidant activities were similar to catechin and superior to BHT. However, EETCc showed higher reactivity than catechin, while the results for the BHT were inconclusive, because the reactivity presented could not be measured by the current protocol.

KEYWORDS: Terminalia catappa; Antioxidant Activity, Catechin

Introduction

Oxygen plays a fundamental role in metabolism of aerobic life forms by catalyzing the energy production at the cells. Nevertheless, this reactions result in the formation of free radicals, also known as ROS and NOS if the unmatched electron is around oxygen and nitrogen atoms, respectively (1). They have some benefits in small amounts, participating in phagocytosis, cellular grown and synthesis of substances; however in excess they interact with other molecules, which can cause oxidative damage to proteins, membranes and genes. Therefore, oxidative stress has been related to many diseases such as arthritis, cardiovascular and neurological conditions and cancer. Furthermore, it has an impact on the body's aging process (2).

Antioxidant compounds or systems are able to neutralize the damage caused by ROS and NOS, reason why there is substantial interest in researching about this subject. Some of them are endogenous, like the enzymes catalase and superoxide dismutase, however most are originated from diet and other sources. Ascorbic acid, tocopherols, polyphenols and carotenoids are examples of the last kind (2).

Food industry has been trying to inhibit lipid oxidation with synthetic free radicals kidnappers. The most common example, the one that was utilized for this study, is the butylated hidroxy toluene (BHT) (3). Studies have shown, however, that these kind of antioxidant possibly have toxic effects. Therefore owing to the suggested harm caused by synthetic substances, researches nowadays are oriented to finding out more about the natural antioxidants.

Medicinal plants are known for containing phenolic compounds. These substances are natural antioxidants because of their ability to react with free radicals forming stable compounds due to the resonance of the aromatic ring in their structure. Many evidences indicate that plants belonging to *Combretaceae* family have revealed antioxidant activity (AA). Among them there is the *Terminalia* gender, which contains plants rich in aromatic substances, including flavonoids, and suggested to have medical properties like antibiotic, anti-inflammatory, antiulcerogenic, anticancer and antifungal activity. *Terminalia pallida*, *Terminalia arjuna* e *Terminalia chebula* have shown AA and antiulcerogenic activity (AUA) in ethanol-induced ulcers. Bark and leaves of *Terminalia brasiliensis* and *Terminalia fagifolia* were found to have AA and AUA (4). In this context, aqueous extract from the leaves of *Terminalia catappa* has also shown AA and liver protection, although there are not many conclusive studies about it, reason why it was chosen to be used in the present study. *Terminalia catappa* grows commonly in the tropical and subtropical countries. The leaf, bark, and fruit of this plant have long been used in folk medicine for antidiarrheic, antipyretic and hemostatic purposes in India, Phillippines, Malaysia and Indonesia. The *Terminalia catappa* leaf has been reported to possess hepatoprotective, anti-inflammatory and anti-HIV reverse transcriptase activity (5).

Polyphenolic compounds in general, particularly the flavonoids, possess the ideal structure for kidnapping free radicals, ability that makes them strong antioxidants (6). Catechins are part of an interest class of flavonoids commonly found in different kinds of food, such as fruits and red wine (7). Besides the antioxidant, they have been proved to have antiallergy and anticancer activity. Catechin was utilized in the present study, since it represents a solid standard for comparison of AA.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods. The odd electron in

the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured (8).

The purpose of the present study is to determine the absorption spectrum of ultraviolet (UV) light and to evaluate the AA of ethanolic extract from the bark of *Terminalia catappa* (EETCc), comparing them with the data obtained from catechin and butylated hidroxy toluene (BHT).

Material and Methods

A single beam spectrophotometer with quartz cuvettes and optical path of one centimeter was the chosen method to determine the UV absorption spectrum (200-400nm). The antioxidant activity was measured by the test with 2,2-diphenyl-1-picrylhydrazyl (DPPH).

For the stoichiometric test stocked solutions of EETCc, catechin and BHT (2,4 mg/mL) were diluted in methanol to 240, 120, 60, 30, 15 and 5 ìg/mL. 200 mL of each solution were added to 2 mL of the DPPH methanolic solution (40 mg/mL) and left resting in ambient temperature. After 30 minutes, the absorbance was measured in 517 nm and utilized to calculate the antioxidant activity (AA%) and the CE_{50} (concentration of antioxidant necessary to reduce in 50% the concentration of DPPH in 30 minutes) (3).

The reactivity of each sample was evaluated in a concentration of 60 mg/mL, and the decrease of the absorbance of DPPH solution was observed in the course of 60 minutes, using the values of absorbance to calculate the residual DPPH percentage and to determine the TE_{50} (time necessary for the initial concentration of DPPH to decrease to 50%).

Methanol (2 mL) in addition to test solution (200 mL) was used as white sample. DPPH solution (2 mL) in addition to methanol (200 mL) was used as control sample. The tests were accomplished in triplicate. The values of CE_{50} and TE_{50} were calculated by non-linear regression.

Result and Discussion

EETCc and catechin, both in 100 mg/mL, have had two peaks of absorption in the spectral region of the UV. The first peak happened in 238 nm for the EETCc **(Figure 1)**, and in 232 nm for the catechin **(Figure 2)**. The second peak happened in approximately 280 nm for both. Similar to cathequin, the EETCc has intense absorption at the C region (200 a 290 nm) and considerably less at the B region (290 a 320 nm) of UV light spectrum.

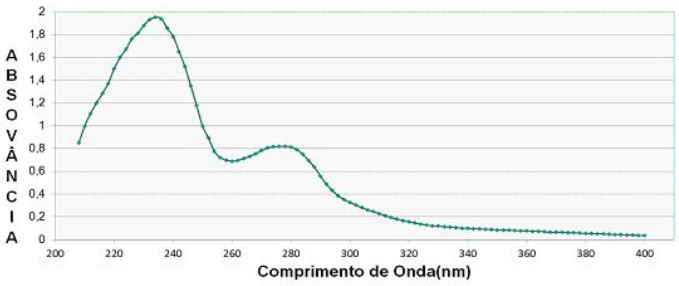

Figure 1: Ultraviolet's absorption spectrum of ethanolic extract from the bark of Terminalia catappa. Teresina, 2011. (Absorvance versus wavelength)

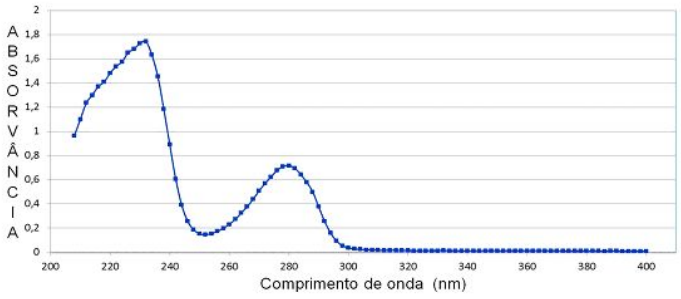

Figure 2: Ultraviolet's absorption spectrum of catechin (2,4mg/mL). Teresina, 2011. (Absorvance versus wavelength)

The study demonstrated that EETCc possesses AA with EC_{50} = 51,1 ± 5,11 µg/mL **(Table 1)**, similar to the catechin (46,3 ± 4,38 µg/mL) and significantly higher

than the BHT (305,7 ± 26,94 µg/mL). However, the EETCc presented higher reactivity (ET_{50} = 22,3 s) than the catechin (ET_{50} = 51,7 s). Results for BHT were inconclusive because the reactivity was not measurable by the current protocol (ET_{50} >60 min).

Extrato / AA	EETCc	Catequina	BHT
EC_{50}	51,1 ± 5,11 µg/mL	46,3 ± 4,38 µg/mL	305,7 ± 26,94 µg/mL
ET_{50}	22,3 s	ET_{50} = 51,7 s	ET_{50} >60 min*

Table 1: Antioxidant Activity (AA) of EETCc, Catechin and BHT (2,4 mg/mL). Teresina, 2011.

* = BHT presented reactivity non-measurable by the current protocol.

Conclusion

The ethanolic extract from the stem bark of *Terminalia catappa* presents antioxidant activities similar to catechin and superior to BHT. However, EETCc showed higher reactivity than catechin, while the results for the BHT were inconclusive.

The research of medicinal plants has a great importance nowadays. There are innumerous plants that, like *Terminalia catappa*, have been studied extensively for their antioxidant and medicinal properties. The study of catechin also has promising results. Data founded suggests an important contribution of catechin in ultraviolet's absortion and in antioxidant activity (AA) of EETCc, since catechin was discovered in the ethanolic extract of many plants that contain AA, including the leaves of *Terminalia fagifolia*.

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