ANTIOXIDANT CAPACITY AND LIPIDIC OXIDATION PROTECTION OF AMAZON ENDOPHYTIC FUNGI EXTRACTS

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ABSTRACT

Endophytic fungi present themselves as a rich source of substances with biotechnological application. Among them are antioxidant compounds, namely phenolic compounds, which can avoid the damage caused by oxidative stress, and minimize the formation of free radicals. Since endophytic fungi isolated from tropical hosts are still poorly studied, we evaluated the global phenolic composition and the antioxidant activity of the metabolites produced by endophytic fungi isolated from the Amazonian medicinal plant *Arrabidaea chica* (crajiru). The fungi were reactivated in PDA and cultivated in liquid medium under static conditions. The metabolites were extracted with ethyl acetate, concentrated, and diluted in ethanol to final concentration of 10 mg/mL. The antioxidant capacity was evaluated by the DPPH, ABTS and FRAP methods. The protection capacity against lipid oxidation was determined for the fungal extract that showed the higher antioxidant capacity. The extract produced by the fungus *Diaporthe cerradensis* CG2-12 (F3) presented the best results of antioxidant capacity: 1560 µmolT/g (DPPH), 2134.04 µmoT/g (ABTS), 1235.08 µmolT/g (FRAP) and 203.44 mg/g of total phenolic content. Regarding protection against lipid oxidation, the plant extract seems to have a better protective effect than the fungal extract, but further studies are necessary to confirm these results.

Keywords: *Diaporthe cerradensis*. Endophytes. Antioxidant capacity. Phenolic compounds. Lipid oxidation protection.

1 INTRODUCTION

In the search for natural compounds with biological activities of interest to different segments of the industry, there is a group of microorganisms which has stood out for producing active substances, as a result of their secondary metabolism [1,2]. These are the endophytic fungi, which inhabit the interior of plant tissues without causing damage to their hosts. These microorganisms maintain a complex relationship with the host that can enable the production of bioactive compounds, which belong to different chemical classes, mainly phenolic compounds [3,4].

Among the bioactive compounds of interest, antioxidants have been targeted, as oxidative stress and the formation of free radicals in the human body are related to several physiological disorders, in addition of being strongly linked to premature skin aging [5,6]. Therefore, antioxidant compounds of natural sources are sought, since they are capable of chemically stabilizing free radicals, minimizing its harmful effects [7]. In this regard, a group of natural substances described in the literature as having antioxidant activity are phenolic compounds. These are classified as secondary metabolites of plants and microorganisms. Its chemical structure consists of at least one benzene ring to which one or more hydroxyl groups are attached. This diverse group is characterized as flavonoids (containing polyphenols) and non-flavonoids (characterized by simple phenols or phenolic acids) [8].

Another type of oxidation of interest, in this case more focused on the food industry, is the lipid oxidation. This has been a very important area as it is related to the alteration of foods, through the production of toxic substances and/or substances that give undesirable flavor and color [9]. This chemical phenomenon occurs naturally and has a direct implication on the functional and commercial value of lipidic raw materials and all products that are formulated from them, whether food, pharmaceutical or cosmetic [10]. In this sense, the objective of this study was to evaluate the antioxidant and protective potential against lipid oxidation of extracts produced by endophytic fungi isolated from the Amazonian medicinal plant *A. chica*, popularly known as crajiru.

2 MATERIAL & METHODS

For this study, three endophytic fungi were used: *Cophinforma mamane* CF2-13 (F1), *Colletotrichum* sp. CG1-7 (F2) and *Diaporthe cerradensis* CG2-12 (F3) which were isolated from the Amazonian plant *A. chica* and are deposited in the Central Microbiological Collection of the Amazonas State University (CCM/UEA). They were reactivated in potato dextrose agar (PDA) and subjected to submerged fermentation in supplemented potato broth (pH 5.0) under static conditions, for 14 days at 30 ºC. The metabolites were extracted with ethyl acetate, and after evaporation of the solvent, the extracts were prepared at a concentration of 10 mg/mL using ethanol P.A. [11-13]. An extract from the host plant (P) was also used for comparison purposes.

The content of total phenolic compounds was determined by measuring the absorbance at 280 nm after diluting the sample with distilled water (1:100) and expressed as acid gallic equivalents (mg/g)[14]. The antioxidant capacity was evaluated using the following methodologies: capture of free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), where 100 µL of the extract and 1900 µL of the DPPH solution were mixed, and after 30 min reaction, the absorbances were measured at 517 nm; capture of the ABTS

radical (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), where 100 µL of the extract was left in contact with 2000 µL of the ABTS solution for 15 min and the absorbances measured at 734 nm; and measurement of the ferric reducing power (FRAP), where 350 µL of the extract and 2450 µL of the FRAP reagent were mixed, and after 30 min the absorbances were read at 595 nm. A Trolox calibration curve was constructed for each method and the curve equation was used to calculate the results, which were expressed in equivalent micromoles of Trolox per gram of extract (µmolT/g) [15,16].

To analyze the protection against lipid oxidation, peroxide values were measured along time: 100 µL of the sample was in contact with 1 g of extra virgin olive oil in closed test tubes for 34 days at two storage temperatures, 20 ºC and 30 ºC. The peroxide index (PI) was measured by titration with 0.1 N sodium thiosulfate at 0, 2, 4, 14 and 34 days, and calculated according to the equation: PI = (Vm x N x 1000)/P, where: Vm = volume in milliliters of the titrant solution; N = normality of the titrant solution; P = weight in grams of the olive oil sample. The results were expressed in milli equivalents of O_2 per kilogram (meqO_2/kg). Finally, in order to evaluate the resulting coloring products of lipids oxidation, the absorbances at 232 nm were obtained. The high absorbance value at this wavelength (≥2.60) may indicate oxidized olive oil. To do this, 0.25 g of the sample was weighed and filled with isooctane in a 25 mL flask [17]. All measurements were performed in triplicate.

The experimental data obtained from antioxidant capacity and total phenols content were submitted to analysis of variance (ANOVA). The software IBM SPSS Statistics v. 29.0.1.0 was used in the statistical analysis of the results ($p < 0.05$).

3 RESULTS & DISCUSSION

The data in Table 1 show the total phenols and the antioxidant capacity, according to each assay method. The extract that presented the highest phenolic content was the one produced by the fungi *Colletotrichum* sp. CG1-7 (F2) with 325.94 mg of acid gallic per g of extract. However, the highest antioxidant capacity was exhibited by the extract from the fungi *Diaporthe cerradensis* CG2-12 (F3), which was observed in the three methods used.

Table 1 Total phenols and antioxidant capacity of host plant (*Arrabidaea chica*) and its endophytic fungi extracts.

Sample	Total phenols (mg/g)*	DPPH (µmol/g)**	ABTS (µmol/g)**	FRAP (µmol/g)**
P	101.57 ^{a} ± 1.99	383.85 ^{a} ± 5.70	418.63 ^{a} ± 6.94	404.43 ^{a} ± 7.79
F1	169.45 ^{b} ± 0.74	190.79 ^{b} ± 2.61	240 ^{b} ± 4.05	415.49 ^{a} ± 3.28
F2	**325.94 ^{c} ± 1.12**	320.11^{c} ± 14.40	2031.76 ^{c} ± 17.36	841.64 ^{b} ± 0.82
F3	203.44 ^{d} ± 1.34	**1560 ^{d} ± 17.28**	**2134.04 ^{d} ± 11.57**	**1235.08 ^{c} ± 22.13**

Values are given as mean of triplicate and standard deviation
Means that do not share a letter are significantly different ($p < 0.05$)
* Values expressed in gallic acid equivalents
** Values expressed in Trolox equivalents

The F3 extract was selected, as it presented the best antioxidant capacity, to evaluate its protective ability against lipid oxidation. The results for 20 ºC and 30 ºC are shown in Figures 1a and 1b, respectively. It is observed that at a storage temperature of 20 ºC, from the 14th day onwards, there are no significant changes between the tests with olive oil alone, and olive oil + F3. However, with olive oil and the plant, there seemed to be a decrease in peroxide index after 24 days of storage, and a kind of pro-oxidant effect after 34 days. For a storage temperature of 30 ºC, peroxide index values tend to have a slight increase, which is expected, perhaps due to the effect of temperature. However, when observing the tests with the plant (A+P) and the fungus (A+F3) extracts, both seemed to have had a slight protective effect regarding the oxidation of olive oil from the 24th day onwards, when compared to the control, containing only olive oil (A).

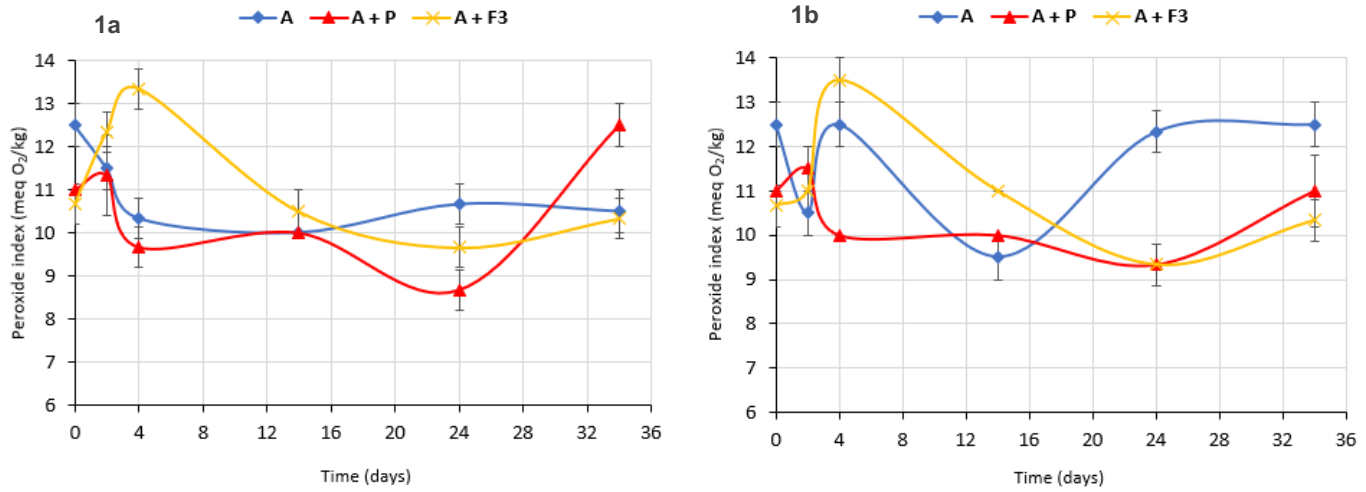

Figure 1 Peroxide Index at 20 ºC (a) and at 30 ºC (b), used to evaluate the protective capacity against lipid oxidation of fungal extract F3 (*Diaporthe cerradensis* CG2-12) and of plant (*Arrabidaea chica*) extract. A = olive oil; A+P = olive oil and plant extract; A+F3 = olive oil and fungal extract.

For absorbances at 232 nm, the results obtained at 20 ºC and 30 ºC are expressed in Figures 2a and 2b, respectively. The storage temperature of 30 ºC continues to favor the oxidation process, although the behavior of olive oil is similar in both temperatures. As for the test with the extracts, at 20 ºC the plant extract obtained lower absorbance values compared to F3 extract. However, within 34 days, both extracts reached very close absorbance values (between 1.80 and 2.00), while in the test at 30 ºC, the plant extract caused a slight decrease in the absorbance value after 34 days, when compared to the F3 extract.

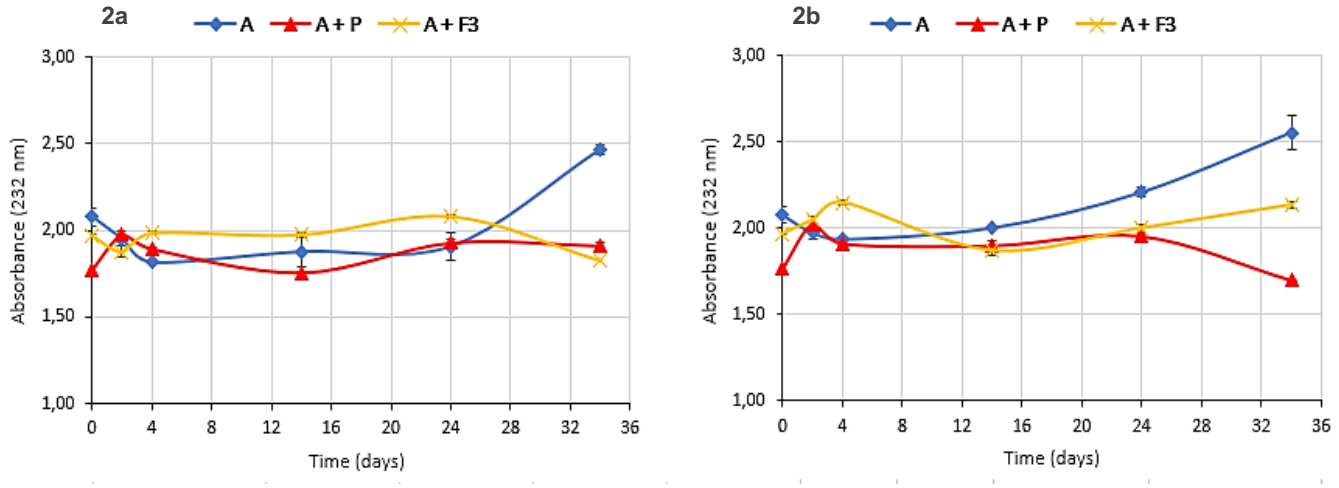

Figure 2 Absorbance values at 20 ºC (a) and 30 ºC (b), used to evaluate the protective capacity against lipid oxidation of fungal extract F3 (*D. cerradensis* CG2-12) and of plant (*A. chica*) extract. A = olive oil; A+P = olive oil and plant extract; A+F3 = olive oil and fungal extract.

Studies evaluating fungal extracts capacity to avoid lipid oxidation are scarce. Generally, it is observed studies using fruit extracts, such as one that evaluated the protective effect of extracts from different types of grapes. In this case, using butter, other authors verified that after 14 days at a temperature of 20 ºC, the control sample (butter only) presented a higher level of peroxides (56.6 meq O_2/kg) while the experiments using the grape extracts, obtained from Touriga Franca, presented a lower value of peroxides (38.1 meq O_2/kg), indicating a potential protective effect against the lipid oxidation[18].

4 CONCLUSION

The extract produced by the fungus *Diaporthe cerradensis* CG2-12 (F3) presented promising antioxidant potential regardless the method used to access the antioxidant activity. However, for a better conclusion about the protective capacity against lipid oxidation and particularly the potential use in food industry, it is suggested to proceed with further experiments.

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