Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Potential of indigenous plants seed extracts of *Anisophyllea boehmii* and *Aframomum sanguineum* from Burundi to protect against oil oxidation

Potencial de extratos de sementes em plantas indígenas de *Anisophyllea boehmii* e *Aframomum sanguineum* do Burundi para a proteção contra a oxidação de óleo

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Abstract

The objective of seed extracts from *Anisophyllea boehmii* and *Aframomum sanguineum* were to evaluate their ability to stabilize against oxidation of oils exposed to sunlight on one hand and subjected to high temperatures on the other hand. Determination of the peroxide value (PV) showed that the extracts had reduced the oxidation of sunflower oils. After 8 weeks of sunlight exposure, the concentration of 265.45 mg/l of *A. boehmii* extract showed a PV of 30.78 meq O_2/kg, 67.4 mg/l extract of *A. sanguineum* had a PV of 42.75 meq O_2/kg while the oils without extracts had a very high PV (125.06 meq O_2/kg). Heating of the oils to 180°C for 8 hours was found, with *A. boehmii* extract (265.45 mg/l), to have a PV of 29.66 meq O_2/kg, with that of *A. sanguineum*, while the PV of the oils without extract reached 50.66 meq O_2/kg. In the light of these results, the seeds of *A. boehmii* and *A. sanguineum* contain antioxydant compounds, which, once extracted, can be used for many purposes in the food processing, pharmaceutical and cosmetic industries.

Key words: *Anisophyllea boehmii*, *Aframomum sanguineum*, peroxide value, stabilizer.

Resumo

O objetivo da extração de sementes de *Anisophyllea boehmii* e *Aframomum sanguineum* foi avaliar sua capacidade de estabilização contra a oxidação de óleos expostos à luz solar, por um lado, e submetidos a altas temperaturas, por outro. A determinação do valor de peróxido (VP) mostrou que os extratos tinham reduzido a oxidação dos óleos de girassol. Após 8 semanas de exposição à luz solar, a concentração de 265,45 mg/l de extrato de *A. boehmii* mostrou um VP de 30,78 ppm O_2/kg, 67,4 mg/l de extrato de *A. sanguineum* tinha um VP de 42,75 ppm O_2/kg enquanto os óleos sem extratos tinham um VP muito alto (125,06 ppm O_2/kg). Aquecimento dos óleos a 180 °C por 8 horas foi obtido com extrato de *A. boehmii* (265,45 mg/l), para obtenção de um VP de 29,66 ppm O_2/kg, com o de *A. sanguineum*, enquanto o VP dos óleos sem extrato atingiu 50,66 ppm O_2/kg. A partir desses resultados, pode-se afirmar que as sementes de *A. boehmii* e *A. sanguineum* contêm compostos antioxidantes que, uma vez extraídos, podem ser utilizados para diversos fins nas indústrias de processamento de alimentos, farmacêutica e cosmética.

Palavras-chave: *Anisophyllea boehmii*, *Aframomum sanguineum*, valor de peróxido, estabilizador.

1. Introduction

Anisophyllea boehmii Engl., with Umusindwi as vernacular name is an wild indigenous species growing in Miombo woodlands (Chidumayo, 1988; Mwakalukwa et al., 2014) which are the most widespread and dominant dry forest formations in Eastern, Central, and Southern Africa (Mwakalukwa et al., 2014). In Burundi, it is found in the natural forests on the Congo Nile ridge and in the fields where they have been deliberately left after clearing. It is also found in Ruvubu National Park. *A. boehmii* bears fruits with edible pericarp (Kalaba et al., 2009). While it provides good firewood, it has poor quality of lumber. Few studies performed on bark and seed revealed respectively an interesting antimalarial bioactivity (Chinsembu, 2015) and an oil content of 29% (Nkengurutse et al., 2019).

Aframomum sanguineum (K. Schumis), with Intake as vernacular name, is a terrestrial rhizomal herbs belonging

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Received: September 27, 2022 – Accepted: December 5, 2022

to the Zingiberaceae family. The Zingiberaceae constitute a family with over 1400 species distributed in over 50 genera (Buruga and Olembo, 1971). The *Aframomum* genus is well represented in lowland rainforest and in many mountain areas. In the East African community, it is frequently used in traditional medicine (Chifundera, 1987; Kamatenesi et al., 2011; Masinde, 2010). In addition, Burundian people have traditionally used its seeds in the preparation of cosmetic anointment (Ngezahayo et al., 2015). Nowadays, they use it as an ingredient in tea and in the rice preparation. Studies have revealed that *A. sanguineum* contains many bioactive compounds. Its essential oil has been reported to contain mono- and sesquiterpenes (Cheikh-Ali et al., 2011) . More than 24 volatile compounds, of which the most abundant were 1.8 cineole (38.5%), a-terpinyl acetate (9.6%), geranyl acetate (9.4%) (Hari et al., 1994)., sabinene (6.7%), have been identified in the seed extract of *A. sanguineum*

Both species,*A. boehmii* and *A. sanguineum,* are characterized by compounds with important bioactivities. However, the investigations were not exhaustive. There are almost no studies performed on antioxidant activity while oxidants are the cause of various diseases: cancer, diabetes, hypertension, coronary heart (Favier, 2003; Lefer and Granger, 2000; Mughal et al., 2024; Spector, 2000; Vicente-Ferreira et al., 2024). Oxidations are also among main factors of deterioration of foods, especially rich in lipids and proteins. Oxidation products are produced from food natural components during manufacture, handling, storage, and culinary preparation (Soladoye et al., 2015). These oxidative products continue the reaction during the succeeding digestion phases, leading to the formation of additional oxidation products with toxic potential (Van Hecke et al., 2016). These potentially harmful chemical species are exposed to the gastrointestinal tract and also to internal organs through intestinal uptake and blood distribution (Estévez and Luna, 2017).

Thus, the overall objective of the present investigation is to valorize the seeds of *A. boehmii* and *A. sanguineum* and more specifically the evaluation of their potential to reduce the oxidation of oils. The results of this study will find wide applications in the food industries especially in the preservation of processed and unprocessed foods, which will contribute to the reduction of diseases of oxidative origin.

2. Materials and Methods

2.1. Origin of plant materials

The seed samples of both species were harvested from three sites of three different eco-climatic zones of Burundi. *A. boehmii* (vernacular name: Umushindwi) samples were collected in the eastern depression (1,200-15,00 m of altitude, 850 mm of precipitation, and 20°C of temperature), the central trays (1,400-2,000 m of altitude, 1,450 mm of precipitation, and 20°C of temperature) and the foothills of Mumirwa (1,000-1500 m of altitude, 1,450 mm of precipitation, and 20°C of temperature). *A. sanguineum* (vernacular name: rutare, urutake) were harvested in the foothills of Mumirwa (1000-1500 m of altitude), the high mountains (1,500-2,600 m), and the central trays (1,400-2,000 m of altitude). The seeds were dried at room temperature and regularly weighed. As soon as the weight became constant, the seeds were ground into powder for subsequent analyses.

2.2. Sample preparation

The first step was the extraction of the oil from the seeds to have oilcake. Seeds were crushed using a Moulinex blinder made in France (Moulinex LM34 *) and then the extraction was performed with hexane as solvent in a Soxhlet apparatus under reflux for 8 h. Then, 50 ml of ethanol (80%) was added to 10 g of completely defatted cake and homogenized for 30 minutes using a magnetic stirrer. Subsequently, the phase separation was done by centrifugation at the 4,000 rpm for 20 min. The supernatant (ethanolic extract) was recovered in a flask and the pellet was again reextracted three times. The three ethanolic extracts obtained were mixed and evaporated to dryness under reduced pressure. The extract was recovered in 50 ml of ethanol 80%. Thus, it allowed to determine its concentration.

2.3. Oxidation kinetics reduction test by A. boehmii and A. sanguineum extracts

2.3.1. Reduction of oxidation kinetics of sunflower oil exposed to sunlight

The first operation was to determine the concentration of extracts to be added to the oil samples. From the ethanolic solution obtained after extraction, 1,000 µl were picked and then the mass of extract contained in 1,000 µl were determined. After, the 1,000 µl were added under rotary evaporator to eliminate the polar solvent immiscible with oil. The apolar solvent (hexane) then recovered the extract. In case the apolar solvent was ineffective, a spatula was used to completely remove the extract.

Secondly, 70 ml of sunflower oil, purchased from Bujumbura Market, were mixed with the solvent containing the extract. Sunflower oil is very rich in polyunsaturated fatty acids (62-70%) (Evrard et al., 2007) and thus characterized by a high degree of oxidation. Then, the solution was submitted rotary evaporator, which allowed to evaporate the polar solvent and to mix the extract and the oil. The mixture was then put in a transparent glass bottle, closed with cap and exposed to sunlight. The experimental period was from May to June, a period when the solar irradiance is around 250 W/m^2 (Lawin et al., 2019). Each week, 5 g were taken to determine the peroxide value for 8 weeks.

2.3.2. Reduction of oxidation kinetics of sunflower oil subjected to heat

100 g were picked from the sunflower oil, purchased from Bujumbura market, and put in a 250 ml glass beaker. All steps leading to the determination of the known concentration of the extracts were followed as described in 2.3.1. Before starting the determination of PVs, the heating was maintained at the recommended temperature of 180°C (Aleena et al., 2020; Farag et al., 2009) for 8 hours,

the peroxide value was determined as the starting zero point. After each hour, the peroxide value was determined during the 7 hours in the oven.

2.3.3. Determination of peroxide value

The peroxide value was determined according to the methodology described in ISO 3960:2007 (ISO, 2007) and it was expressed in milliequivalents of active oxygen (meq O_2)/kg of oil.

2.4. Statistical analysis

Data analysis was performed using IBM SPSS statistic 20. Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlation between various parameters was also investigated. Significance was determined at $p < 0.05$ level and the results were expressed as mean values ± standard deviation (SD). All tests were performed in triplicate.

3. Results and Discussion

3.1. Extraction yield

The extraction yields of the extracts from the seeds of *A. boehmii* and *A. sanguineum* are depicted in Table 1. Between the two species, *A. boehmii* yield (92.91±23.62 g/kg) was found to be very significantly ($p < 0.05$) different from that of *A. sanguineum* (23.62±0.98 g/kg). This is normal because the species are different and therefore the composition and hardness are different. These results are lower than those found, with the same methods and same solvent, in the species of *Entada abyssinica* Steudel ex A. Rich, *Annona senegalensis* Pers, *Brachystegia longifolia* Benth, *Caesalpinia decapetala* (Roth) Alston, *Dodonaea viscosa* Jacq, *Ipomoea involucrata* P. Beauv, *Myrianthus arboreus* Beauv., *Maesopsis eminii* Engl, *Parinari curatellifolia* Planch. Ex Benth, *Sterculia tragacantha* Lindl, *Tephrosia vogelii* Hook.f., *and*, *Uvaria angolensis* Welw. ex Oliv (Niyukuri et al., 2020). Although the seeds are harvested from different agro-ecological zones, the yields of the same species showed slight significant ($p < 0.05$) differences. Thus, for the *A. boehmii* extracts , the order in terms of extraction yield is as follows: Central trays (98.29 ± 4.07 g/kg) > Eastern depression (93.33±5.18 g/kg) > Foothills of Mumirwa (87.12±7.11g/kg). With *A. sanguineum* extract, the extraction yield order is: Foothills of Mumirwa (26.01±3.44 g/kg) > Central trays (24.04±0.98 g/ kg) > High mountains (20.82±4.53 g/kg). This small difference may be due to the different climatic conditions that make the proportion of the accumulation of elements differ from one place to another.

Table 1. Extraction yield of extracts from the *A. boehmii* and *A. sanguineum* seeds (expressed in g of extract per kg of oilcake).

	A. boehmii	***A. sanguineum***
Eastern depression	93.33±5.18^{b}	N/A
Central trays	98.29±4.07^{a}	24.04±0.98^{ab}
Foothills of Mumirwa	87.12±7.11^{ab}	26.01±3.44^{a}
High mountains	N/A	20.82±4.53^{b}
Average	92.91±23.62^{u}	23.62±0.98^{v}

N/A = Not Applicable. The letters a and b in superscript, in the columns, show significant differences between samples of the same species from different sites while u and v, in the last row, show significant differences between species.

3.2. Reduction of the oxidation kinetics of oil exposed to sunlight by extracts from A.boehmii and A. sanguineum seeds

Photo-oxidation, an important process in the weathering of oil, produces a variety of oxidized compounds, including aliphatic and aromatic ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxides, phenols, anhydrides, quinones and aliphatic and aromatic alcohols (Lee, 2003). Among these products formed by photo-oxidation, some are very harmful to health and are the cause of various fatal diseases. Epoxides are able to cause biological effects with genetic mechanisms: point mutations, deletions, chromosomal aberrations, gene conversion, crossing-over, cancer and virus (prophage) induction (Ehrenberg and Hussain, 1981; Manson, 1980).

In this study, it was shown the potential reduction of sunflower oil oxydation by extracts from *A. boehmii* and *A. sanguneum* (Figures 1-2). Before the use of the extracts, the Peroxide Value (PV) of the oils purchased at the market was determined. The PV obtained was 4.21±0.84 meq O_2/kg and was found to be similar to 4.19 meq O_2/kg reported by Konuskan et al. (2019).

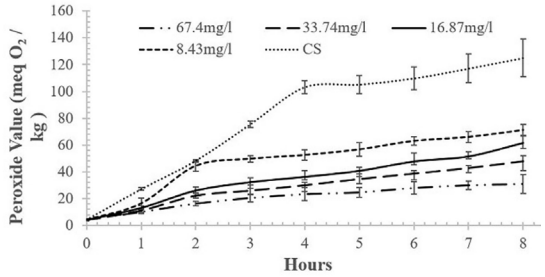

Figure 1. Reduction of the oxidation of the oil subjected to the sun by the extract of A. sanguinum (CS: control sample).

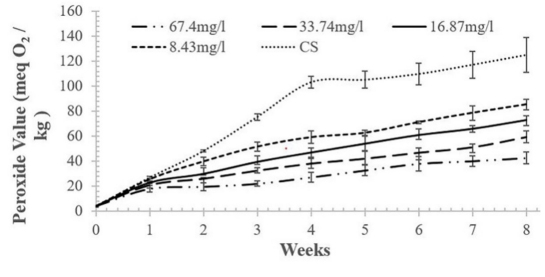

Figure 2. Reduction of the oxidation of oil subjected to the sun by the extract of *A.sanguineum* (CS: control sample).

For *A.boehmii* extract, using the concentration of 265.45 mg/l (Figure 1) the PV was 16.23±1.7 meq O_2/kg of oil after two weeks of exposure to sunlight. At this same time interval, with the concentration of 132.72 mg/l, the PV has reached 22.16±2.07 meq O_2/kg whereas the extract concentration of 66.36 mg/l corresponded with the PV of 26,069± 2.50 meq O_2/kg of oil. Through this study, it was realized that even low concentrations showed a reduction in the dynamics of oxidation. The concentration of 33.18 mg/l managed to stabilize the PV at 44.49 ±3.90 meq O_2/kg while the oxidation in the control oil (without extract) reached 48.28±0.85 meq O_2/kg. According to CODEX STAN 210-1999 standards (FAO, 1999), refined oils < 10 meq O_2/kg/kg oil while cold pressed and virgin oils < 15 meq O_2/kg /kg oil. Furthermore, CODEX STAN 33-1981 norms (FAO, 2009) fixed on virgin olive oils the PVs ≤ 20 meq O_2 / kg).

After 4 weeks of exposure to the sun, the PVs from the extracts concentrations of 265.45 mg/l, 132.72 mg/l, 66.36 mg/l, and 33.18 mg/l were respectively 23.25±4.80; 29.93±3.43; 36.27±4.80; 52.64±3.76 meq O_2/kg. This being so while control samples, the PVs had reached 103.25±4.80 after two weeks: a very significant difference ($p < 0.05$) compared to the PVs of oils with extracts. For oil containing no extract, the PV varies very slowly from the first week to the 8th week. This suggests that the oxidation is complete; there are no more polyunsaturated fatty acids but full of peroxides.

In comparison with the PVs of the oils without extracts (controls), we realize that, at the 8th week, the oils with *A. boehmii* extracts still contain polyunsaturated fatty acids. While the oil containing the less concentrated extract showed a PV of 71.22±4.20 meq O_2/kg, that of the more concentrated extract 30.78±2.28, the oil without extract had 125.06±14.01 meq O_2/kg. Whether it is the PV of the extract less concentrated, whether it is more concentrated compared to the PV of the oil without extract, there is always a very significant difference ($p < 0.05$). In addition, PVs of oils containing the most concentrated extracts are not very far from 20 meq O_2/kg fixed by CODEX STAN 33-1981 norms (FAO, 2009). Thus, *A. boehmii* extracts can stabilise oils against oxidation due to sunlight.

The *A. sanguineum* extract has also been tested on the reduction of oxidation dynamics (Figure 2). Oils with extract concentrations of 67.4 mg/l, 33.74 mg/l, 16.87 mg/l, and 8.43 mg/l have been exposed to the sun during 8 weeks. Their PVs were respectively compared with the PVs of control oils containing no extracts.

After two weeks of sunlight exposure the PVs of the oils were respectively and in ascending order of concentration: 19.74±3.32, 26.16±0.83, 30.15±4.91, and 39.94±3.53 meq O_2/kg. At this same exposure time, the PV of the control oil already had values reaching 48.28±0.85 meq O_2/kg. It can be seen that the PV of the oil containing a high concentration of *A.sanguineum* is similar to that (20 meq O_2/kg) fixed by CODEX STAN 33-1981 norms (FAO, 2009) for virgin olive oil.

After 4 weeks, the PV of a control sample (103.25±4.80 meq O_2/kg) was twice that of the sample (59.26±4.9 meq O_2/kg) containing a very low concentration of *A. sanguneum* while the one with the high concentration had a PV of 27.27±3.80 meq O_2/kg. This shows that the extract strongly reduced the rate of oxidation of sunflower oils.

On the 8th week, the PVs of the different concentrations (67.4 mg/l, 33.74 mg/l, 16.87 mg/l, and 8.43 mg/l) were respectively 42.75±4.58, 59.39±4.89, 73.29±3.20, 85.46±40 with significant differences at $p<0.05$. Compared to the PV of the control sample, we find that even after two months the extract preserves the oils from oxidation by the sun. According to these results we can affirm without hesitation that even low doses of the extracts associated with the conservation of the oils away from sunlight can preserve the oils from oxidation for a long period.

3.3. Reduction of the oxidation kinetics of oil subjected to high heat by extracts from A.boehmii and A. sanguineum seeds

In culinary preparations, we often apply high temperatures. In almost all the villages of Burundi, there is proliferation of donuts manufacture by heating the oils at high temperatures, while in the cities, in addition to the donuts, many fries are prepared, especially in the cabarets. Studies performed on flaxseed oil have reported that the polyunsaturated fatty acid content have significantly ($p < 0.05$) decreased by 5.75 µg/g after heating at 210 °C (Sun et al., 2021). During this investigation, extracts of *A. boehmii* and *A. sanguneum* at different concentrations were added to sunflower oil and then subjected to 180°C for 8 hours (Figure 3). Before the heating (at time 00h00), the PV was 4.21±0.84 meq O_2/kg. It the same value that it was found for the experiment with sunlight before exposing (at time of 00h00).

After 2 hours, the results of the *A.boehmii* extracts with the concentrations of 265.45 mg/l, 132.72 mg/l, 66.36 mg/l, and 33.18 mg/l (same concentration of the previous section) revealed PVs not significantly different ($p> 0.05$) for the concentrations that follow each other and order is as: 11.66±4.04 > 15.00±3 > 18.66±3.51 > 21±4 meq O_2/kg. Compared to control samples (CS), we see that the lowest concentration has a lower PV, therefore reducing the kinetics of oxidation. As shown in the results, if the concentration is halved, the VPs are not halved. This means that once the antioxidant compound is extracted, a very low concentration would be efficient. After 4 hours of heating, the same trends observed at the 2nd hour were

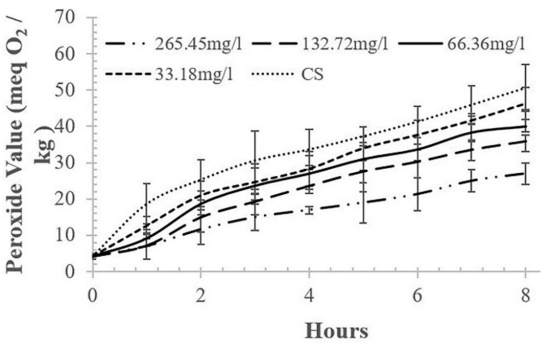

Figure 3. Reduction of oxidation of oil subjected to 180°C by the extract of A. boehmii (CS: control sample).

observed. While the highest concentration resulted in a PV of 17.01 ±6.05 meq O_2/kg, the lowest was 28.33 ±4.80 meq O_2/kg and the control 32.45±1.55 meq O_2/kg. No significant differences (p> 0.05) between two concentrations that follow each other.

After 8 hours under the temperature of 180°C, the concentration of 265.45 mg/l, 132.72 mg/l, 66.36 mg/l, and 33.18 mg/l resulted in maintaining the PVs respectively at 29.66 ±3.04, 33.04 ±2.11, 38.1.22±3.60 and 42. ±2.50 meq O_2/kg. However, no significant difference was observed between the less concentrated sample and the control samples (50.66±2.52 meq O_2/kg). This means that with *A.boehmii* extracts, any ambient temperature during conservation cannot affect the oil quality by oxidizing it when contain that extract.

The experiment also focused on the extracts of *A. sanguineum* (Figure 4). It was found that after two hours of heating at 180°C, the concentrations of 67.4 mg/l, 33.74 mg/l, 16.87 mg/l, and 8.43 mg/l, respectively stabilized the oils from oxidation at the following PVs: 14.2.11±2.56 > 17.33±5.50 > 21.66±3.03 > 24.5±2.92 meq O_2/kg. Still no significant differences (p> 0.05) between two consecutive concentrations. Unlike previous experiments, the lowest concentration recorded a PV almost equal to that of the control samples (25.33 ±5.50 meq O_2/kg). However, that does not mean that the extract does not contain antioxidant compounds, it is a matter of concentration.

After 4 hours of heating, the results show that the polyunsaturated fatty acids are not yet completely oxidized. While the VPs of high concentrations amounted to 19.03±2.11 meq O_2/kg, those of low concentrations were 32.81±1.55 meq O_2/kg. For the same duration, the extract of the control sample had a PV, which reached 33.66±5.59 meq O_2/kg. Even after eight hours, the bioactive principles were still functional. Always characterized by the lack of significant (p> 0.05) difference between the PVs of two consecutive concentrations. While the control sample had a PV of 50.66±6.50, the oils containing the *A. sanguneum* extracts (67.4 mg/l, 33.74 mg/l, 16.87 mg/l, and 8.43 mg/l) were at 29.66± 3.04, 33.01±2.07, 38.42±3.60, 42.33±2.50 meq O_2/kg. Although the oil containing a more concentrated extract has a PV above 20 meq O_2/kg fixed by CODEX STAN 33-1981 norms (FAO, 2009), if the antioxidant components are purified, they will be more effective even at low concentrations. Thus, they could be used in the food processing industries as a preservative ingredient, especially since the population in their food preparations already uses them. Indeed, the seeds of *A. sanguineum* are sometimes added to rice preparations, and when finely ground, they are added to tea, not only to give a pleasant smell and taste much appreciated by urban dwellers in Burundi and neighboring countries, but also as a medicine to prevent throat infections (Hari et al., 1994).

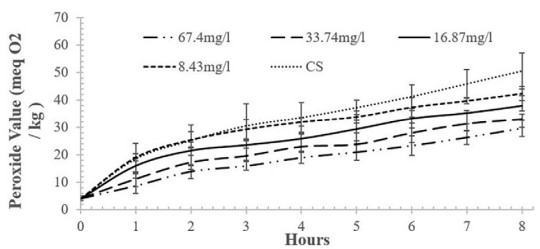

Figure 4. Reduction of oxidation of oil subjected to 180°C by the extract of A. sanguineum (CS: control sample).

4. Conclusion

The present investigation has demonstrated the ability of extracts from *A. boehmii* and *A. sanguneum* to slow down the oxidative deterioration of the sunflower oil. The extract of both species were found to have the potential to reduce the oxidation kinetics of sunflower oil exposed to sunlight. It was also shown that these extracts have the potential to stabilize oils subjected to high temperatures during cooking process such as baking, frying and fritters. This suggests that the seeds of these two species contain antioxidant compounds, which, once purified, could be used in the agri-food and pharmaceutical industries.

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