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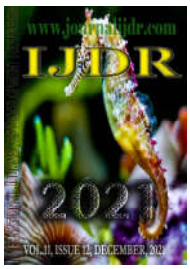

RESEARCH ARTICLE OPEN ACCESS

EVALUATION OF THE ANTITUMOR ACTIVITY OF COLD-PRESSED OIL OF DIPTERYXALATA VOGEL ALMONDS WITH POTATO DISC BIOASSAY

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ABSTRACT

Research on Cerrado species and bioproducts is an important means of disseminating biological resources, aiming at the conservation of the biome and contributing to the dissemination of knowledge about benefits for human health. Therefore, the use of natural products has been the subject of investigations in several to assess the beneficial potential of bioproducts. In this paper, the antitumor effect against strains of *Rhyzobuimradiobacter* was investigated using the potato disc methodology. The cold-pressed oil of *Dipteryxalata(D. alata)* almonds, rich in unsaturated fatty acids, presented promising results that allow us to conclude that there was a significant inhibition of the tumoral activity of *R. radiobacter*.

Citation: ***Elizabeth de Fátima Lopes da Rocha, Isadora Nascimento Fernandes, Jhenys Sara Sardinha Shimokawa et al.*** "Evaluation of the antitumor activity of cold-pressed oil of Dipteryxalata Vogel almonds with potato disc bioassay*", International Journal of Development Research*, 11, (12), 52911-52914.

INTRODUCTION

Dipteryxalata almond oil has been used in folk medicine for the antiveninous treatment of *Bothropsjararacussu* (surucucu) stings, being also important hepato and cardioprotective Ribeiro *et al.,* (2014). The pharmaceutical and cosmetic industry is interested in the economic potential of *D. alata*oil as it contains high levels of phytosterols that can be used as vehicles for high-quality drugs and cosmetic agents Moraes*et al.*, (2018). A study by Esteves and collaborators (2011) evaluated the cytotoxic and genotoxic potentials of *D. alata* highlighting the low number of scientific publications with plants considered medicinal. The term "*medicinal plants*" refers to plant elements that aim to alleviate or cure diseases and have their tradition of use as medicine based on the popular knowledge of a community Brazil (2012).

The oil of *D. alata* presents high contents of unsaturated fatty acids such as oleic, linoleic and linolenic acids recognized, according to the scientific literature, as antimutagenic substances with antiproliferative role (Oliveira-Hohne, 2013; Marques *et al*., 2015; Oliveira-Alves *et al*., 2020). The term antimutagenic refers to the ability to reduce the frequency of mutations by any compound, natural or synthetic (Sloczynska *et al*., 2014). The bacterium *Rhyzobiumradiobacter* causes rapid proliferation in some plant species, culminating in tumor formation. Because of its nucleic acid composition and histology similar to animal and human cancers, it is used as a biotest for new antitumor compounds (Silva *et al*., 2018). Bioassay tests play a relevant role in the exploration of clinically useful antitumor agents. The potato (*Solanum tuberosum*) disc bioassay makes it possible to verify antitumor properties of biological and synthetic bioactives by being fast, cheap, simpleand reliable (Coker *et al*., 2003). Is a gram-negative bacterium, natural soil inhabitant, responsible for causing

tumors in plants and opportunistic infections in humans (Marta *et al*., (2011). The present research aimed to observe and quantify the antitumor effect of *D. alata* almonds oil on potato discs inoculated with *R. radiobacter*.

MATERIALS AND METHODS

The da assay was performed using the method adapted from Coker *et al*., (2003) and Nge (2016). The potatoes were purchased fresh, medium to large size and healthy, in the Central Market of Anápolis, Goiás. The *D. alata* oil used in the bioassay has IBD (Biodynamic Institute Certification Association) certification for natural products. IBD certification has an international reputation and is supervised by institutions such as IFOAM (*International Federation of Organic Agriculture Movements*) from England and Demeter International, in Germany (IBD, 2021).

a

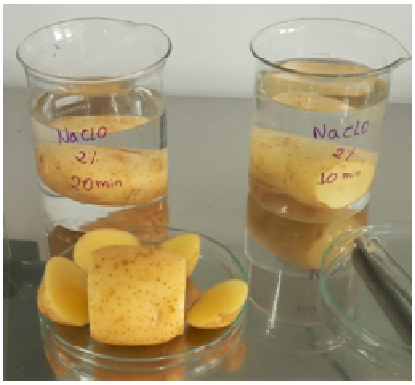
b

Source: Own authorship, 2021.

Figure 1. Potatoes (a) and *D. alata* oil (b) obtained for the biotest

In the laboratory, the potatoes were washed with water and dried. Afterwards, they had their surfaces disinfected by immersion in 0.1% sodium hypochlorite solution for 20 minutes (Figure 2-a).

a



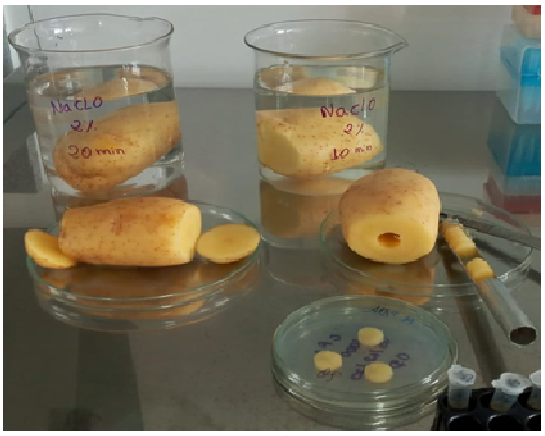
b

c

Source: Own authorship, 2021

Figure 2. Decontamination of the potato, removal of the ends, cutting and preparation of the discs

The potato ends were removed followed by immersion in 0.1% sodium hypochlorite solution for another 10 minutes (Figure 2-b). A 10 mm cylindrical tissue core was extracted from each potato with the aid of a sterile metallic drill. At the end of each cylindrical cores, 20 mm pieces were removed and then discarded (Figure 2-c). With a sterile scalpel, 5 mm thick discs were removed from the remaining cylindrical core. Subsequently, three potato discs were deposited in Petri plates containing bacteriological agar, which had received the addition of the *R. radiobacter* bacterium inoculum prepared together with the oil from *D. alata* almonds (Figure3).



Source: Own authorship, 2021.

Figure 3. Preparation of the potato discs in the *Petri* dishes

The bacterium *R. radiobacter* cultivated in Tryptone Soy Agar (TSA) medium for 48 hours at a temperature of 25 ºC (Figure 4). Then 3 to 4 typical and determined strains were removed, and with these a suspension was prepared compared to a 0.5 McFarland scale ($1{,}5x10^{8}$ $UFC.mL^{-1}$) in TSB tryptone soy broth which was incubated for 48 hours at 25 °C.

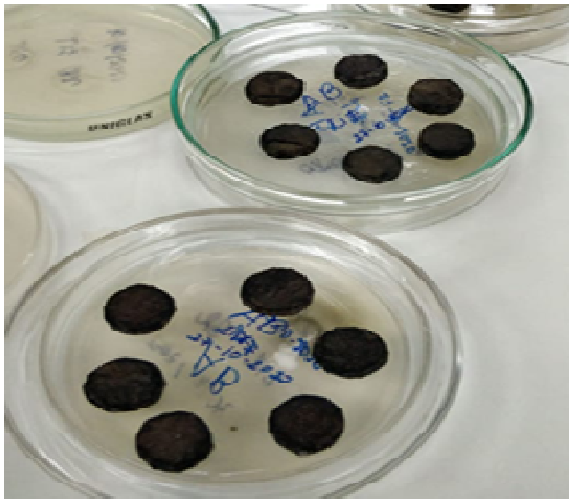
Then, the inoculum was readjusted again to 1,5x10^{8} UFC.mL^{-1}with a 0.5 McFarland scale and a 1:100 dilution was carried out, obtaining an inoculum with 1,5x10^{6} UFC.mL^{-1}.

Source: Own authorship, 2021.

Figure 4. Bacterium *R. radiobacter cultivated for 48 hours on bacteriological agar*

The oil from *D. alata* almonds was dissolved in 5% dimethylsulfoxide (DMSO) and in 0.02% TSB broth of Tween 80®and diluted accordingly with 2000 µl.mL^{-1};1000 µl.mL^{-1}; 500 µl.mL^{-1}; 250 µl.mL^{-1}; 125 µl.mL^{-1}; 62,5 µl.mL^{-1}with the bacterial culture suspension, leaving it to be incubated for 30 minutes at room temperature, with subsequent homogenization forthe inoculation of 50 µL in the potato discs. As a positive control, a camptothecin was used, dissolved in 5% DMSO in the operations of 1 µl.mL^{-1} , 10 µl.mL^{-1} , 100 µl.mL^{-1} e 500 µl.mL^{-1} bacterial suspension (Coker *et al*., 2003; Trigui*et al.,* 2013; Nge, 2016).

The potato discs were incubated for 20 days at 25 °C and after this period they were read with a Lugol's solution (5% I2 and 10% KI), the tumor counts were performed and compared with the controls (Figure 5). The results were presented in percentage in the following formula:$R\% = \frac{NTC-NTT}{NTC} \times 100$, onde R% is result in percentage, NTT is "treatment tumor number" and NTC "control tumor number."



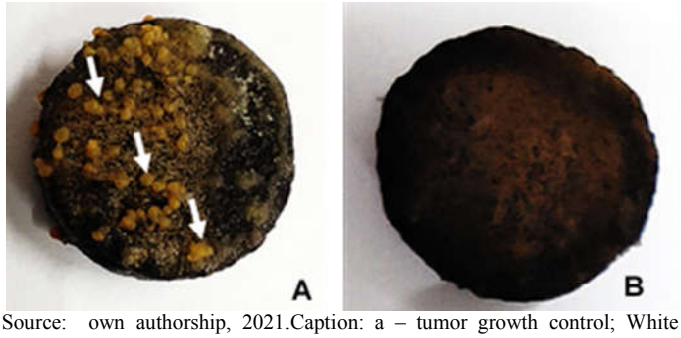
Source: Own authorship, 2021.

Figure 5. Potato discs after staining with Lugol

Significant tumor inhibition activity was considered when values were consistent with 20% or greater inhibition. The data obtained for testing the antitumor activity of *D. alata* kernel oil on potato discs were submitted to a one-way analysis of variance (ANOVA) with significance level p < 0.05 performed in Office Excel 2020.

RESULTS AND DISCUSSION

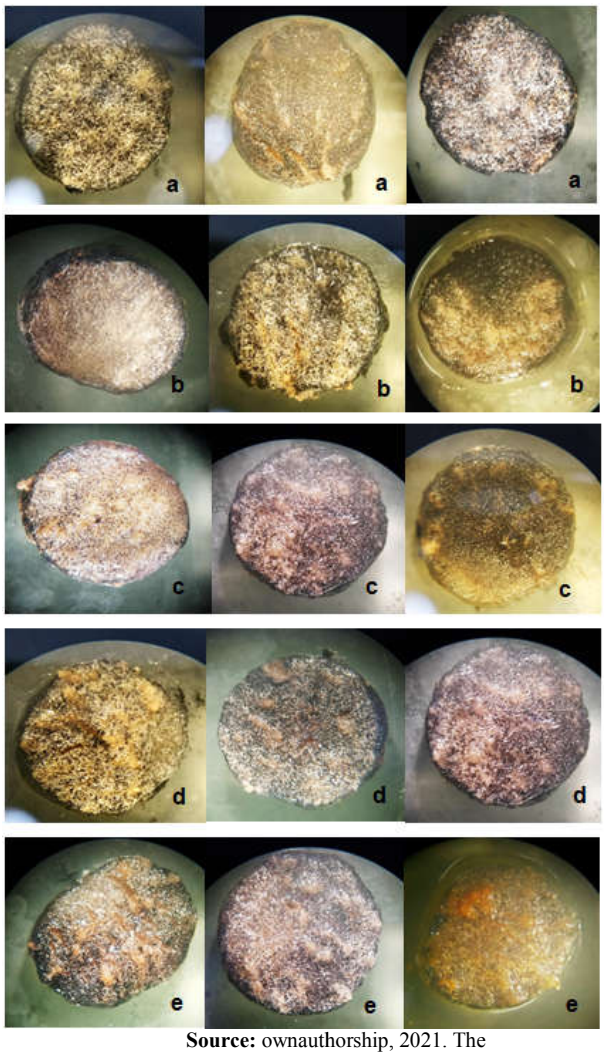
The bioassay using potato discs was done in triplicate. Where Figure 6-a shows the tumor growth control (CCT) with the presence of tumor and Figure 6-b shows the positive control with Camptothecin which shows free tumor development.



Source: own authorship, 2021.Caption: a – tumor growth control; White arrows pointing to tumors and b - positive control with camptothecin.

Figure 6. Presence and absence of tumors in the control tests

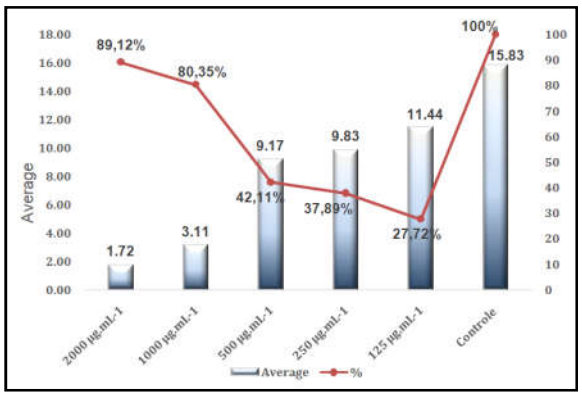
The results of the antitumor activity with the oil of the almonds of *D. alata* were observed with better performance in the concentrations of 2000 µl and 1000 µl. From the 500 µl concentration on, the tumor count, but the values were still within the valid percentage for antitumor inhibition. Figure 7 shows the three phases of the test with the respective applied concentrations.



Source: ownauthorship, 2021. The lettersidentifytheconcentrationsandtheirtriplicates: **a** (2000µg.mL), **b** (1000µg.mL), **c** (500µg.mL), **d** (250µg.mL) and**e** (125µg.mL).

Figure 7. Sequence of photos corresponding to the three steps of the bioassay

As presented in the images in the previous figure, tumor formations were significantly inhibited in the presence of *D. alata* oil where it was in higher concentrations. The potato disc bioassay method has been used for the past 15 years and adapted for the purpose of standardization or control of bioactive components (Yousif *et al.*, 2012). The effects of tumor inhibition after the days elapsed from the application of the concentrations of *D. alata* oil are represented below (Figure 8).



Source: Own authorship, 2021.

Figure 8. Percentage of the antitumor inhibition factor of *D. alata* oil

CONCLUSION

The efficacy of antitumor action was directly proportional to the increase in oil concentration used. The results of the present investigations proved to be encouraging and significant regarding the *in vitro* antitumor activity of *D. alata* almonds oil on potato discs. Further studies are needed to identify which bioactive compounds in the oil were of greatest relevance. With further proof, *D. alata* oil could be a source of potent tumor agents for drugs in development.

Acknowledgment

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