REVISTASAPIÊNCIA
sociedade, saberes e práticas educacionais | 2238-3565 |

SCREENING OF THE ANTI-TUMOR ACTIVITY OF THE ESSENTIAL OIL FROM INFLORESCENCE OF *Tridax procumbens*

Triagem da atividade antitumoral do óleo essencial das inflorescências de Tridax procumbens

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Resumo: *Tridax procumbens* é uma espécie botânica comumente conhecida por suas propriedades medicinais atribuídas à presença de constituintes químicos específicos que possuem propriedades terapêuticas e profiláticas contra diversas doenças, incluindo a ação antitumoral. Com base nessa hipótese, foi conduzida a prospecção fitoquímica preliminar dos óleos voláteis das inflorescências de *T. procumbens* com o propósito de se investigar a atividade antitumoral por meio da técnica de inibição da formação de tumores em discos de batata pela bactéria *Rhizobium radiobacter* ATCC 4720. Amostras vegetais das inflorescências de *T. procumbens* foram coletadas para extração dos óleos voláteis por arraste a vapor utilizando a técnica de hidrodestilação por Clevenger. A prospecção fitoquímica foi realizada utilizando a técnica de marcha analítica com o material pulverizado da inflorescência de *T. procumbens*. A caracterização química foi realizada utilizando cromatografia gasosa acoplada a espectrometria de massas. A atividade antitumoral foi avaliada em discos de batata pela observação da inibição da formação de tumores por *R. radiobacter*, nesse teste foram avaliadas diferentes diluições do óleo (125, 250, 500, 1000 e 2000 $\mu g.mL^{-1}$). A prospecção fitoquímica revelou a presença de flavonoides, cumarinas, heterosídeos cardioativos, esteroides e saponinas. Nas análises do óleo volátil foram identificados 26 compostos químicos, sendo o decano 2,4,6-trimetil o composto majoritário. A atividade antitumoral apresentou resultados significativos a partir da menor concentração testada, com uma inibição de 63,8% na formação de tumores nos discos de batata. Portanto, verificamos que o óleo volátil de *T. procumbens* apresentou potencial antitumoral de maneira dose dependente, com inibição significativa da formação de tumores no ensaio com discos de batata.

Palavras-chave: Erva-de-touro. Prospecção fitoquímica. Atividade antitumoral. Ensaio de discos de batata. *Rhizobium radiobacter*.

Abstract: *Tridax procumbens* is a botanical species commonly known for its medicinal properties attributed to the presence of specific chemical constituents that have therapeutic and prophylactic properties against various diseases, including antitumor action. Based on this hypothesis, a preliminary phytochemical prospection of the volatile oils from *T. procumbens* inflorescences was conducted with the purpose of investigating the antitumor activity with the technique of inhibition of tumor formation on potato discs by the *Rhizobium radiobacter* ATCC 4720. Plant samples from *T. procumbens* inflorescences were collected for extraction of volatile oils by steam-

dragging using the Clevenger hydrodistillation technique. Phytochemical prospection was carried out using the analytical march technique with the pulverized material from the inflorescence of *T. procumbens*. Chemical characterization was performed using gas chromatography coupled with mass spectrometry. The antitumor activity was evaluated in potato discs by observing the inhibition of tumor formation by *R. radiobacter* with different concentrations of the volatile oil (125, 250, 500, 1000 and 2000 $\mu g.mL^{-1}$). The phytochemical prospection revealed the presence of flavonoids, coumarins, cardioactive heterosides, steroids and saponins. In the volatile oil analyses, 26 chemical compounds were identified, with 2,4,6-trimethyl decane being the majority compound. The antitumor activity showed significant results from the lowest concentration tested, with a 63.8% inhibition of tumor formation in potato discs. Therefore, we verified that the volatile oil of *T. procumbens* showed antitumor potential in a dose dependent manner, with significant inhibition of tumor formation in the assay with potato discs.

Keywords: T*ridax* daisy. Phytochemical prospection. Antitumor activity. Potato disc assay. *Rhizobium radiobacter*.

INTRODUCTION

Tridax procumbens, popularly known as tridax daisy, coat buttons, or mexican daisy is widely used in folk medicine to treat various health problems (STEFANELLO et al., 2018). The plant has anti-inflammatory and analgesic properties and is commonly used to treat gastrointestinal problems, fever, and headaches (RIBEIRO et al., 2018). Additionally, other scientific studies have shown that *Tridax procumbens* has antitumor potential (TADDEI; ROSAS-ROMERO, 2000; CERQUEIRA et al., 2002).

Chemical compounds present in the plant, such as flavonoids and terpenoids, have been the target of studies and present antitumor activity (LIMA et al., 2020). For example, extracts from *T. procumbens* were able to inhibit the proliferation of breast and colon tumor cells in culture (SREELATHA; PADMA, 2009) and the plant was able to reduce the growth of prostate tumors in mice (RIBEIRO et al., 2018).

Screening for antitumor potential based on the inhibitory activity of nodule formation in *Rhizobium radiobacter*-infected potato discs is a convenient and inexpensive alternative to detect antitumor activity of plant extracts (BINNS; THOMASHOW, 1988). Tests with known plant extracts showed similar results to those obtained with commercially available pharmaceutical substances (FERRIGNI et al., 1982; TRIGUI et al., 2013). In addition, the antitumor activity of *Tridax procumbens* components does not affect the bacterial viability of *R. radiobacter*, and it is possible to test bacterial viability using a plate with specific culture medium (MCLAUGHLIN; ROGERS; ANDERSON, 1998).

In summary, *T. procumbens* is a plant with important medicinal properties, especially in the treatment of tumors. Chemical compounds present in the plant have been the target of

studies and may serve as a basis for the development of new antitumor therapies. In view of the above, this work studies the main chemical constituents present in the volatile oil of the inflorescence of *Tridax procumbens* and its potential for tumor inhibition by potato disc assay.

METHODOLOGY

Plant Material

Samples of inflorescence of *T. procumbens* were collected in the Central Park Senador Onofre Quinan in Anápolis, Goiás, Brazil, in the geographical coordinates 16° 20' 19" S 48° 57' 53" O, from 7am to 8am, during the month of March 2019. These samples were prepared in the Biology Laboratory of the Central Campus Anápolis of the State University of Goiás, where exsiccates were made, which were identified by Professor Mirley Luciene Santos, curator of the University Herbarium and deposited under the tomb number 12438.

The samples were dried in an oven with air circulation and renewal (Solab SL-102) at 40 °C for 48 hours, and then pulverized in a Marconi MA580 knife mill. The granulometry of the tamis was 0.85 mm, the powder was stored at room temperature and dry, away from light until use.

Phytochemical prospection

After treatment and pulverization of *T. procumbens* inflorescence, the samples were submitted to characterization screening of the most relevant secondary metabolites, separated into three major groups: phenolic compounds (simple phenols, coumarins, flavonoids, anthraquinones and tannins); nitrogen compounds (alkaloids) and terpenes (cardioactive heterosides and saponins). They were submitted in characterization reactions for phytochemical prospection in triplicates, with methodology adapted from Matos (2009) and Simões et al. (2017).

Extraction of the volatile oil

The extraction of the volatile oil from the inflorescence of *T. procumbens* was performed by the hydrodistillation method, using Clevenger apparatus. For this, 100 g of the dried and ground sample was used in 1,000 mL of distilled water coupled to the Clevenger apparatus and kept on a heating mantle until boiling (approximately 100 ºC) for three hours (Figure 1). HPLC ethyl ether was used for the complete removal of the volatile oil from the Clevenger apparatus.

The extracted volatile oil was dried with anhydrous sodium sulfate (Na_2SO_4), and stored in an amber bottle with absolute seal and under refrigeration (-20 ºC) (BARROS et al., 2018).

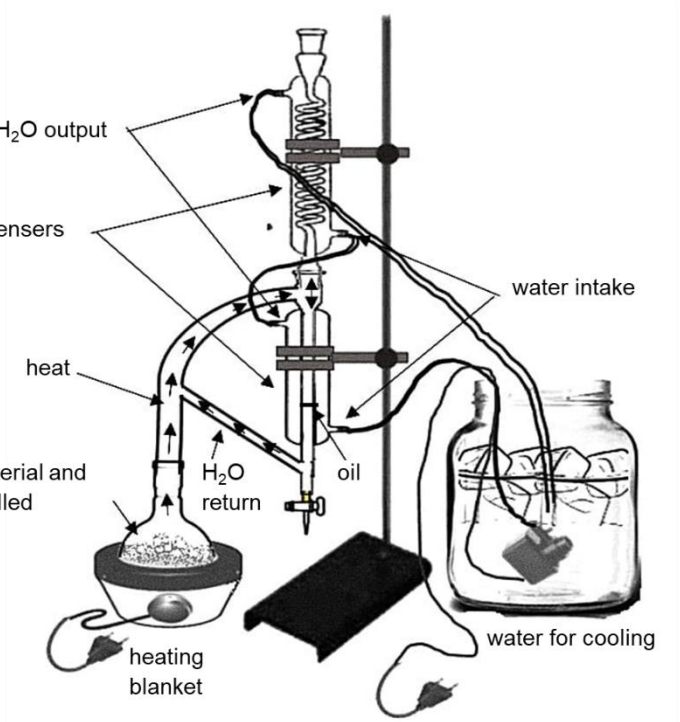


Figure 1 - Extraction of volatile oil from *T. procumbens*

To obtain the yield in percent volatile oil, a calculation was made based on the mass of oil obtained in relation to the mass of plant material used on a moisture-free (BLU) basis (SANTOS et al., 2004).

Employing the equation: $$\Re\% = \frac{VOE}{Bms - \frac{Bm - U}{100}} \times 100$$

Where:

RE% = Extraction yield in percent,

VOE = Volume of oil extracted (mL),

Bm = Extracted plant biomass,

U = Moisture content,

Bms = Dry plant biomass,

100 = Conversion factor to percent.

To calculate the yield of the extracted volatile oil, it is necessary to determine the moisture content of the plant biomass, in which the gravimetry method is used in greenhouses (BRASIL, 2010). It was submitted 2.0 g of the pulverized sample for drying in an oven at 103 ± 2°C, in crucible previously dried at the same temperature, until reaching the stabilization of weight after three consecutive weighing, a condition that characterizes the complete absence of water. The procedure was performed in triplicate.

Characterization of the volatile oil

The volatile oil obtained from the extraction process was submitted to gas chromatographic analysis coupled to mass spectrometry (GC/MS) in a SHIMADZU GCMS-QP2010 ultra device. The sample used in the chromatography was prepared at a concentration of 1:10. The oil dissolved in HPLC grade hexane, with the injection of 1µL in the RTX-5MS column of 30 cm length and 0.25 mm internal diameter, using helium gas for dragging at a flow rate of 1 mL/min, with an initial temperature of 60 °C for 2 min. Then the temperature was increased to 240 °C at a rate of 3 °C/min^{-1}. Then it was increased until it reached 280 °C at a rate of 10 °C/min^{-1} maintaining this temperature for 10 min and ionization energy of 70 eV. The injector temperature was 250 °C and operated in Split Less: 20 mode.

Compounds were identified by computer database using the NIST11/2011/EPA/NIH digital mass spectrum data library and by comparison with their retention indices and authentic mass spectra reported in the literature for the most common volatile oil components (ADAMS, 2017). A co-injection of a C_8-C_{32} hydrocarbon mixture (Sigma-Aldrich) was performed as a standard and used the arithmetic index calculation according to the equation of (VANDENDOOL; KRATZ, 1962) described as follows:

$$IR= 100.N[(tx - tn)/(tn - tn\text{-}1)]+100.Cn - 1$$

Where:

N = Cn - Cn-1

Cn = number of carbons of the n-alkane eluting after the analyte.

Cn-1 = the number of carbons in the n-alkane eluting before the analyte.

tx = retention time of the analyte.

tn = retention time of the n-alkane eluting after the analyte.

tn-1 = retention time of the n-alkane eluting before the analyte.

Screening for antitumor activity

Screening of the antitumor activity of the volatile oil from *T. procumbens* inflorescence was performed using the potato (*Solanum tuberosum*) disc assay, with minor modifications of the methods of COKER et al., 2003 and TRIGUI et al., 2013. Briefly, fresh medium-sized potatoes were purchased from a local market and sent to the laboratory for processing. Initially, the potatoes were washed with soap and water and immersed in 0.1 % sodium hypochlorite solution for 20 minutes for surface disinfection, wiped dry on sterilized paper towels. Soon after, about 20 mm of the ends of the potatoes were removed and cylinders of the internal tissues of the disinfected potatoes were extracted with a sterile metal drill. The cylinders were immediately rinsed in sterile distilled water and after discarding the ends, 5 mm thick by 10 mm diameter discs were obtained, which were also rinsed in sterile distilled water, the discs were dried on sterile paper towels and placed in a Petri dish with 2% bacteriological agar (Figure 2).

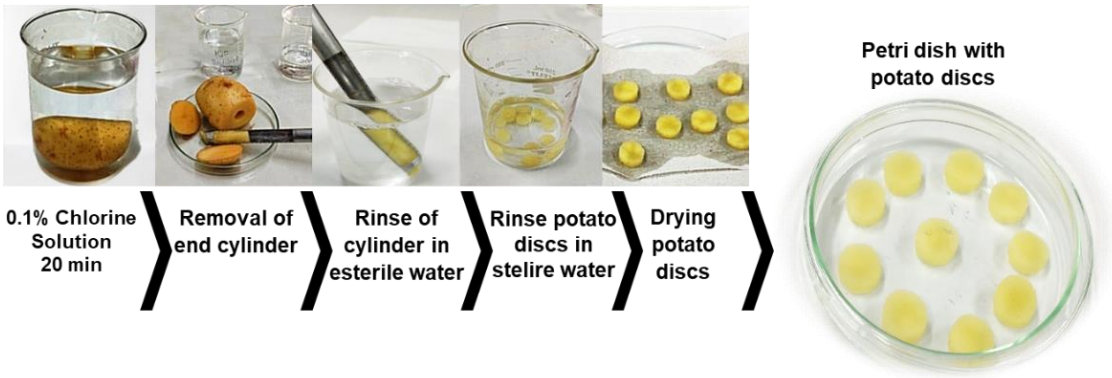


Figure 2 - Preparation of potato discs for testing

Potato discs were inoculated with the bacterial suspension of *R. radiobacter* and concentrations of the volatile oil from the inflorescence of *T. procumbens*. Bacterial suspensions of *Rhizobium radiobacter* ATCC 4720 were prepared by transferring isolated and typical colonies grown on tryptone soy agar (TSA) for 48 h at 25 ºC to tubes with tryptone soy broth (TSB). Then, the turbidity of the suspensions was visually adjusted with McFarland's 0.5 scale to approximately 1.5 x 10^8 CFU.mL^{-1}. Subsequently, the bacterial inocula were exposed

to concentrations of 2000, 1000, 500, 250, 125 and 62.5 µg.mL^{-1} of the volatile oil of *T. procumbens* dissolved in TSB broth with 5% DMSO and 0.02% tween 80® for 30 minutes at room temperature. Then, 50 µL of the samples were homogenized and inoculated onto the potato discs (Figure 3).

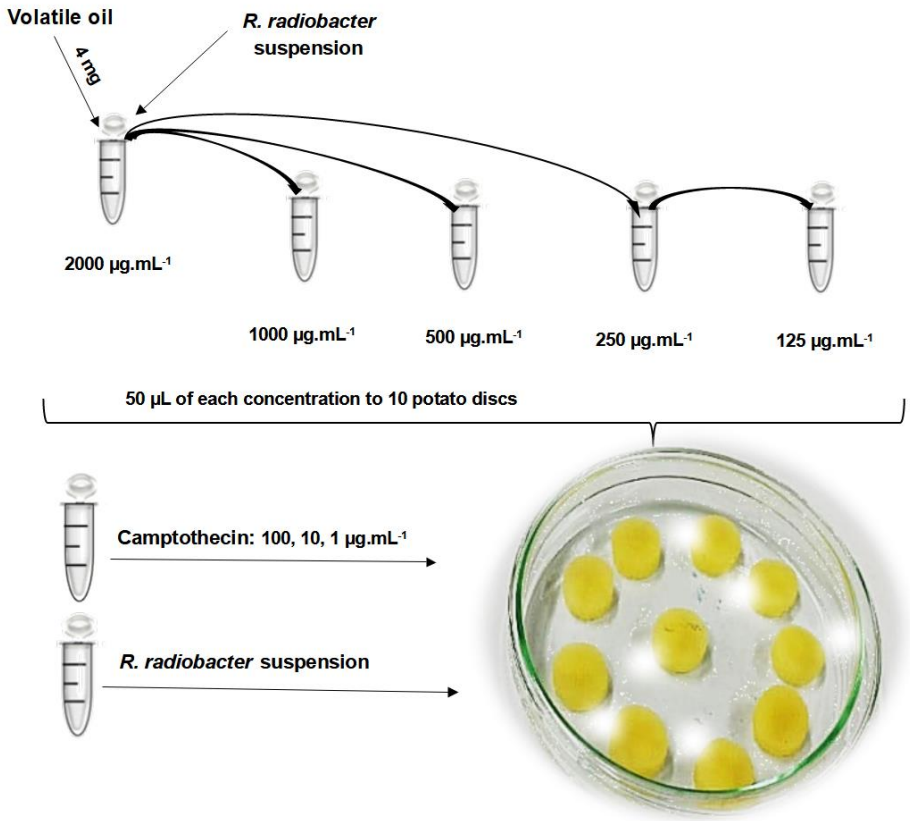


Figure 3 - Screening for antitumor activity with the potato disc assay

Tumor growth controls were included with inoculation of 50 µL of the bacterial suspension not exposed to the volatile oil of *T. procumbens*, technique control with exposure of the bacterial inocula to camptothecin at concentrations of 100, 10 and 1 µg.mL^{-1}. Controls with uninoculated potato discs not treated with any substance were included in the assay.

Bacterial viability was checked by inoculating 100 µL of each suspension exposed to concentrations of *T. procumbens* oils in Petri dishes with TSA incubated at 25 ºC for 48 h to make sure that the volatile oil did not inhibit the growth of *R. radiobacter*.

The potato discs were incubated for 21 days at 25 °C. After this period, tumors formed by *R. radiobacter* were counted on the potato discs after staining with lugol solution (5% I_2 and 10% KI) for 1 minute and then the discs were analyzed under a binocular stereomicroscope (Leica) to count the tumors. The experiment was performed in triplicates with 10 discs for each condition evaluated.

The results were calculated with the following formula:

$$R\% = (100-NTT)/NTC\times100,$$

Where:
R% is result in percentage,
NTT is number of tumors observed in the treated samples and
NTC the number of tumors observed in the untreated controls.

RESULTS AND DISCUSSION

Phytochemical prospection

The reactions of the phytochemical prospection of the pulverized material from the inflorescence of *T. procumbens* indicated positive results for several secondary metabolites (Table 1).

TABLE 1 - Phytochemical prospection of the inflorescences of *T. procumbens*

Phytochemical	**Reactions**	**TpI**
Phenolic Compounds		
Flavonoids	Shinoda	Positive
	Oxalo-Boric	Positive
	H_2SO_4 concentrate	Positive
Phenolic Compounds in general	Alkaline hydroxides	Positive

	$AlCl_3$	Positive
	$FeCl_3$	Positive
Anthraquinonic heterosides	Indirect Bornträger	Positive
Coumarins	NaOH	Positive
Tannins	Gelatin	Negative
	Indirect Bornträger (Quinine)	Negative
	Alkaloids (Quinine)	Negative
	Metal salts ($Cu_2(OAc)4$)	Negative
	Metal salts ($FeCl_3$)	Negative
Nitrogenous Compounds		
Alkaloids	Reagent of Mayer	Negative
	Reagent of Dragendorff	Negative
	Reagent of Bouchardat	Negative
	Reagent of Bertrand	Negative
	Reagent of Hager	Negative
	Tannic acid	Negative
Terpene Compounds		
Heterosides Cardioactive/Steroid	Liebermann-Burchard	Positive
	Pesez	Positive
	Keller-Kiliani	Positive
	Kedde	Positive

Saponins	Foam Index (IE)	<100

Note: TpI - *T. procumbens* inflorescence.

The results indicate that the inflorescence of *T. procumbens* are rich in terpenic and phenolic compounds, such as flavonoids and coumarins, also cardioactive heterosides that are considered important secondary metabolites due to their various biological properties. The presence of saponins at a concentration below 100 in the foam index indicates that these compounds are also present in the sample, but in smaller quantities (BRASIL, 2010). The absence of tannins and alkaloids is important for the safety of the medicinal use of this plant, since these compounds can be toxic in high concentrations (PADUCH et al., 2007).

Furthermore, the results of the antitumor activity of the volatile oil extracted from *T. procumbens* on potato discs are encouraging. The significant inhibition of tumor formation at all concentrations tested indicates that the plant may be a potential source of antitumor compounds. Importantly, the results obtained on potato discs cannot be directly extrapolated to humans or animals, but provides an initial indication of the potential activity of the plant.

Yield and characterization of volatile oil constituents

The yield of volatile oil extraction from *T. procumbens* was 1.51% and the oil was characterized as a slightly yellowish liquid with low viscosity and characteristic odor. The chemical composition of the volatile oil was mainly composed of terpenes, including monoterpenes and sesquiterpenes, with the main components identified as 2,4,6-trimethyldecane (37.83%), α-ionone (10.42%), 2-hydroxymethylhexan-1-ol (9.30%), undecanal (6.18%), and tetrahydrogeraniol (6.16%) (Table 2).

TABLE 2 - Chemical constituents of volatile oil from *T. procumbens* inflorescence

T	IR	Name of compound	MM	AR%	Formula
13.360	1121	2,4,6-Trimethyldecane	184	37,83	$C_{13}H_{28}$
13.990	1134	Tetrahydrogeraniol	186	6,16	$C_{10}H_{22}O$
14.138	1138	2-hydroxymethylhexan-1-ol	136	9,30	$C_7H_{16}O_2$
20.005	1263	Decylenic alcohol	156	4,25	$C_{10}H_{20}O$

21.275	1296	(R) - (+) - Citronellic acid	170	2,90	$C_{10}H_{18}O_2$
24.414	1369	Undecanal	170	6,18	$C_{11}H_{22}O$
25.230	1388	Trans-β-Damascenone	190	0,57	$C_{13}H_{18}O$
25.579	1071	Formic acid	136	1,47	$C_8H_8O_2$
25.790	1428	Dictamnol (none)	178	3,90	$C_{12}H_{18}O$
27.031	1431	α-ionona	192	10,42	$C_{13}H_{20}O$
35.205	1641	Bisaboladienol	208	2,55	$C_{15}H_{26}O$
34.135	1608	β-atlantol	220	1,69	$C_{15}H_{24}O$
29.795	1499	α-Himalachene	204	0,73	$C_{15}H_{24}$
36.586	1678	Cyclotetradecane	196	1,29	$C_{14}H_{28}$
37.434	1700	n-heptadecane	240	0,46	$C_{17}H_{36}$
38.430	1730	Methylisonanoate	254	1,51	$C_{16}H_{30}O_2$
45.509	1849	2-heptadecanona	254	2,10	$C_{17}H_{34}O$
48.420	2034	Farnesyl butyrate	292	0,60	$C_{19}H_{32}O_2$
52.543	2172	Trans-oleic acid (octadocenoic))	282	3,44	$C_{18}H_{34}O_2$
53.330	2196	Stearic acid (ethyl octadecanoate)	312	1,04	$C_{20}H_{40}O_2$
56.183	2300	Tricosan	324	1,61	$C_{23}H_{48}$

Note: T- Retention time; MM - Molar mass; AR% - Relative abundance; IR- Retention index.

The quality and composition of the volatile oil can vary due to several environmental factors, such as the part of the plant sampled, geographical location of the harvest, collection time, cultivation method, climate, age of the plant material and its own genetics informations (MATOS, 2009; SIMÕES et al., 2017).

In addition, the storage conditions of the plant material, as well as the method and duration of extraction of the volatile oil can also affect the yield and quality of the oil. Short extraction times may not extract all components, while long times may lead to degradation of some components (PÉRINO-ISSARTIER et al., 2013; TRAPP; CROTEAU, 2016).

The chemical components are arranged in order of elution according to the chromatogram of the GC/MS analysis. The majority compounds belong to the hydrocarbon, acetone and ester groups. The presence of fatty acids such as oleic acid and stearic acid were

identified, which have applications in the food and pharmaceutical areas, for their antioxidant properties exploited in the prevention of degenerative diseases (OLIVEIRA, 2013).

The available literature has shown volatile compositions with the predominance of monoterpenes and sesquiterpenes in other species of the Asteraceae family (FABIANE et al., 2008), while, (MANJAMALAI; MAHESH KUMAR; BERLIN GRACE, 2012) reported that there is a predominance of monoterpenes and sesquiterpenes in the composition of the volatile oil of the leaves of *T. procumbens*. The majority compound 2,4,6-trimethyldecane is an alkane present in other aromatic species such as the Indian-origin mung bean, *Vigna radiata* (L.) Wilczek (ATTAR et al., 2017).

Citronellic acid or citronellol is a natural acyclic monoterpene present in the oil of cardamom (*Elettaria cardamomum*), rose (*Rosa* sp.), geranium (*Chrysanthemum* sp.), citronella and other species. It has applications as flavoring agents in food products and cigarettes. Repellent, larvicidal, antibacterial properties are described. In addition, citronellol and farnesyl derivatives have the ability to interfere with postprandial hyperglycemia. Tetrahydrogeraniol is an acyclic monoterpene, whose flavoring property is appreciated by the food industry (VALDES; CALZADA; MENDIETA-WEJEBE, 2019).

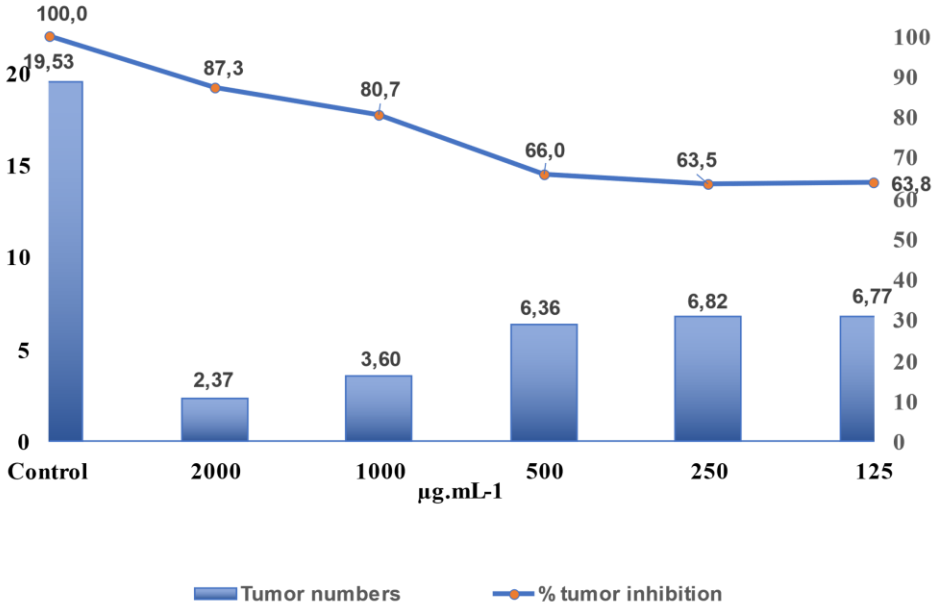
The compound trans-damascenone is a nor-isoprenoid also identified in wine obtained from *Vitis vinifera* and responsible for its strong aroma (YUAN; QIAN, 2015). The β-atlantol is a monocyclic sesquiterpene present in the volatile oil of the species *Piper aequale*, whose antioxidant and cytotoxic properties were demonstrated by Silva et al. (2016) and in *Ocotea diospyrifolia* (Meisn.) Mez. in studies developed by (FABRI et al., 2019).

Therefore, the volatile oil of the inflorescence of *T. procumbens* presents itself promising for the realization of investigations aimed at the search for flavorings and condiments, as well as, in the isolation of compounds with biological potential.

Antitumor activity of volatile oil from *T. procumbens* inflorescence

The results obtained with the number of tumors and percentage of inhibition of tumor induction by *R. radiobacter* are shown in Graph 1.

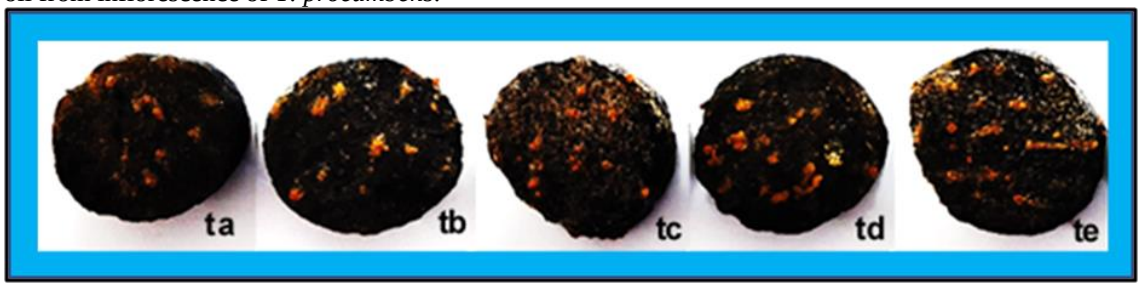
Graph 1 - Percentage of inhibition of tumor formation on potato discs by the volatile oil of inflorescence of *T. procumbens.*



The volatile oil showed significant inhibition of tumor formation on potato discs at all concentrations tested. The results we found in this study for the volatile oil from *T. procumbens* were significant in inhibiting tumor formation on potato disc by *R. radiobacter* ($P<0.0001$).

Similar results were found at concentrations of 125, 250 and 500 µg.mL^{-1}, with inhibition percentages of 63.8%, 63.5% and 66%, respectively (Figure 4 - panels tc, td and te). Higher concentrations of 1000 and 2000 µg.mL^{-1} were able to further reduce tumor formation on potato discs, with inhibition percentages of 80.7% and 87.3%, consecutively (Figure 4 - panels ta and tb).

FIGURE 4 - Formation of tumors by *R. radiobacter* on potato discs in the presence of concentrations of volatile oil from inflorescence of *T. procumbens*.



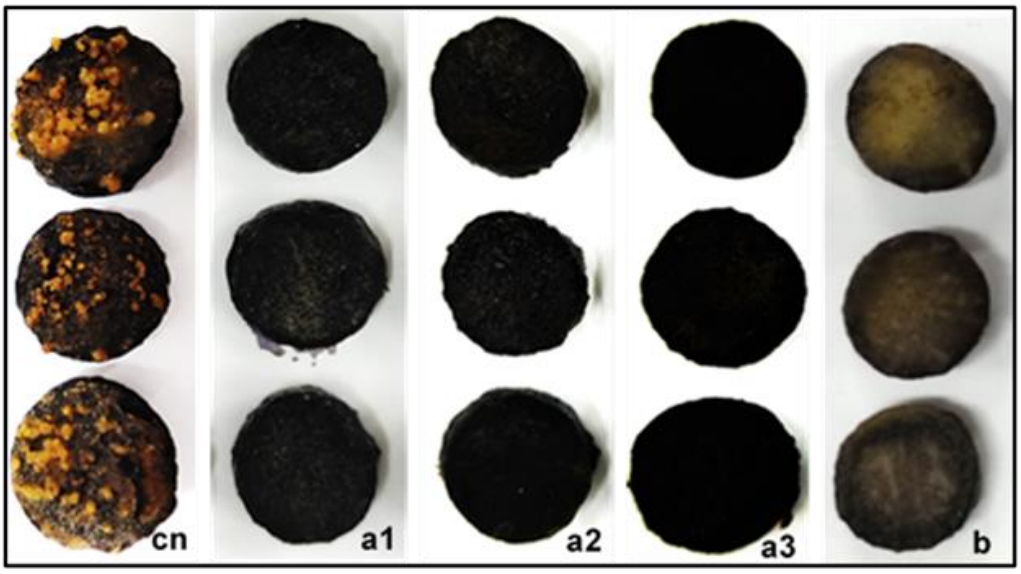
Note: ta - 2000, tb - 1000, tc - 500, td - 250, te - 125. All concentrations are express in µg.mL^{-1}.

Tumor formation percentages were calculated relative to untreated viability controls, which had an average tumor formation of 19.3 mm, corresponding to 100% tumor formation, as shown in Figure 5 (panel cn).

The concentrations of the volatile oil from *T. procumbens* inflorescence tested did not affect bacterial viability during treatment, as evidenced by the positive growth results of the inocula on TSA.

Furthermore, potato discs containing camptothecin at concentrations of 1, 10 and 100 $\mu g.mL^{-1}$ (Figure 5 a1, a2 and a3) and uninoculated potato discs (Figure 5 b) showed no growth, indicating the antitumor activity was not due to inhibition of bacterial growth but rather in blocking tumor formation by the tested compounds.

Figure 5 - Controls of the potato discs assay with *R. radiobacter*



Note: a1 – Camptothecin 100 $\mu g.mL^{-1}$, a2 - Camptothecin 10 $\mu g.mL^{-1}$, a3 - Camptothecin 1 $\mu g.mL^{-1}$. b - uninoculated potato disc, cn - negative control.

The results obtained in this study are in line with other research that investigated the effect of essential oils on tumor cells. Filho et al. (2020) observed significant results of inhibition of breast cancer tumor cells with the use of *Eremanthus erythropappus* essential oil, as well as tumor inhibition is dependent on the concentration used. Similarly, Padilha et al. (2020) conducted studies with squamous neoplastic cells of human uterine cervical and reported an expressive inhibition of tumor cell viability with the use of essential oil from a plant species of the Asteraceae family.

The use of plants with preventive antitumor activities is an important strategy to fight cancer (KALIA, KATNORIA, and NAGPAL 2016). However, many plants still lack data available in the literature, making it necessary to search for antitumor activities using appropriate pharmacological methods (COKER et al., 2003). A difference equal to or greater than 20% in volatile oil treated discs compared to the count in untreated discs is considered significant in antitumor activity (TRIGUI et al., 2013).

Although the mechanism of action of essential oils is not yet completely elucidated, it is known that their cytotoxic activity can be attributed to several sites of action in cells, since they have a complex chemical composition. In addition, essential oils have a lipid character that favors their entry into cells, making them more permeable and leading to the loss of ions and other cellular contents (BAKKALI et al., 2008).

CONCLUSIONS

The phytochemical analysis of the volatile oil extracted from the inflorescence of *T. procumbens* revealed the presence of 21 chemical compounds, including monoterpenes and sesquiterpenes, which are known for their important biological properties. In addition, the study verified the antitumor potential of the volatile oil, with a significant inhibition of tumor formation in potato discs at different concentrations, proving the dose-dependent relationship.

Our results indicate that the exposure of potato discs to the volatile oil of *T. procumbens* showed an inhibitory action against tumor formation by the bacterium *R. radiobacter*, indicating a possible activity against the formation of different types of tumors, fact that should be proven by further studies with cell lines, animal models, and clinical screenings.

Moreover, the cytotoxic action of the volatile oil cannot be attributed to a single mechanism of action, as it has a complex chemical composition that can act on several cellular sites. Thus, it is necessary to continue prospecting for new compounds with relevant biological activities in order to advance the treatment and prevention of different types of cancer.

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