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Bioprospecting the *Cerrado*'s Aromatic Flora: Chemical and Biological Studies of Three Essential Oils

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HIGHLIGHTS

- The sesquiterpene germacrene D was the major constituent in the three EOs under investigation.
- EO from *Campomanesia adamantium* was considered active against *Malassezia furfur*.
- All EOs exhibited *in vitro* anti-inflammatory activity by the chemotaxis model.
- The most abundant metabolite class of all EOs under study was formed by sesquiterpene hydrocarbons.

Abstract: Chemical and pharmacological potentials of botanical species found in the *Cerrado* are well-known and widely studied. Chemical diversity of secondary metabolites produced by plants that belong to this Brazilian biome has triggered several studies in the fields of farming, pharmaceutical and cosmetic industries. Therefore, this study aimed at evaluating the chemical composition of essential oils (EOs) from fresh leaves of three species found in the Brazilian *Cerrado*: *Cardiopetalum calophyllum* Schltdl. (EO-CC), *Campomanesia adamantium* (Cambess.) O. Berg (EO-CA) and *Protium ovatum* Engl. (EO-PO) and at determining their anti-*Malassezia furfur* and anti-inflammatory activities. Both GC-FID and GC-MS showed that the most abundant chemical class of the oils was the one of sesquiterpene hydrocarbons. The major constituents identified in EO-CC were germacrene D (34.9%) and bicyclogermacrene (26.8%). EO-CA exhibited mainly germacrene D (21.7%) while EO-PO had high concentrations of germacrene D (25.0%) and γ-muurolene (18.6%). EOs were tested by the broth microdilution method on 96-well microplates and exhibited satisfactory activity against *M. furfur*, i. e., EO-CA had MIC = 375 µg/mL while EO-CC and EO-PO

had MIC = 750 µg/mL. The chemotaxis model, which was used for evaluating their anti-inflammatory activity, showed that EOs exhibited effective results: *C. calophyllum* (EO-CC; IC_{50} = 24.4 µg/mL), *C. adamantium* (EO-CA; IC_{50} = 15.7 µg/mL) and *P. ovatum* (EO-PO; IC_{50} = 32.5 µg/mL). In short, biological activities of EO-CC, EO-CA and EO-PO, such as anti-*Malassezia furfur* and anti-inflammatory ones, were investigated and described for the first time.

Keywords: *Cardiopetalum calophyllum*; *Campomanesia adamantium*; *Protium ovatum*; *Malassezia furfur*; chemotaxis model.

INTRODUCTION

The *Cerrado* has been known as one of the biomes with the highest biodiversity in the world. The biome stretches over approximately two million km^2, which represents about 23% of the Brazilian territory [1]. Its plants have adapted to distinct environmental conditions, such as long droughts, high precipitation periods, poor soil, frequent fire occurrence and high UV radiation. As a result, plants need to use mechanisms of defense to protect themselves against physical, chemical and biological agents throughout their evolutionary process. This fact may be associated with their bioactive compounds [1].

Since several plants native to the *Cerrado* are aromatic and major sources of bioactive essential oils (EOs), many researchers have focused on the search for promising compounds. EOs are chemical compounds that act in self-defense and attraction of pollinators. Plants produce EOs in their flowers, fruit skin (citric ones), leaves, little grains, roots, bark, bark resin and seeds [2].

The species *Cardiopetalum calophyllum*, which belongs to the Annonaceae family, is very common in the *Cerrado* that stretches over Goiás (GO) state, Brazil. It has been known as *imbirinha* and its EOs have already been evaluated against phytopathogenic fungi, such as *Sclerotinia sclerotiorum*, and have had their antioxidant and antibacterial activities investigated [3]. Besides, *Campomanesia adamantium* (Myrtaceae) has been used by folk medicine to treat hypertension, rheumatism and diabetes. Its common names are *guavira*, *gabiroba* and *guabiroba* [4]. Other studies have also described its antibacterial, antioxidant, anti-inflammatory, anti-diarrheal and urinary antiseptic activities [5]. *Protium ovatum,* whose common name is *vick-do-cerrado,* belongs to the Burseraceae family and is quite promising but few studies of its properties have been carried out. They describe the chemical composition of EOs from some parts of the plant and highlight their anti-*Trypanossoma cruzi*, cytotoxic, antifungal and antileishmanial activities [6-8]. It is worth mentioning that antiproliferative activity of EOs from *C. calophyllum* (EO-CC), *C. adamantium* (EO-CA) and *P. ovatum* (EO-PO) have also been recently reported [9].

Therefore, since our research group is interested in plants native to the *Cerrado* [10] due to their richness in biological properties, this study aimed at analyzing the chemical composition of EOs from *C. calophyllum* (EO-CC), *C. adamantium* (EO-CA) and *P. ovatum* (EO-PO) (Figure 1) leaves and at describing their *in vitro* anti-*Malassezia furfur* and anti-inflammatory activities for the first time.

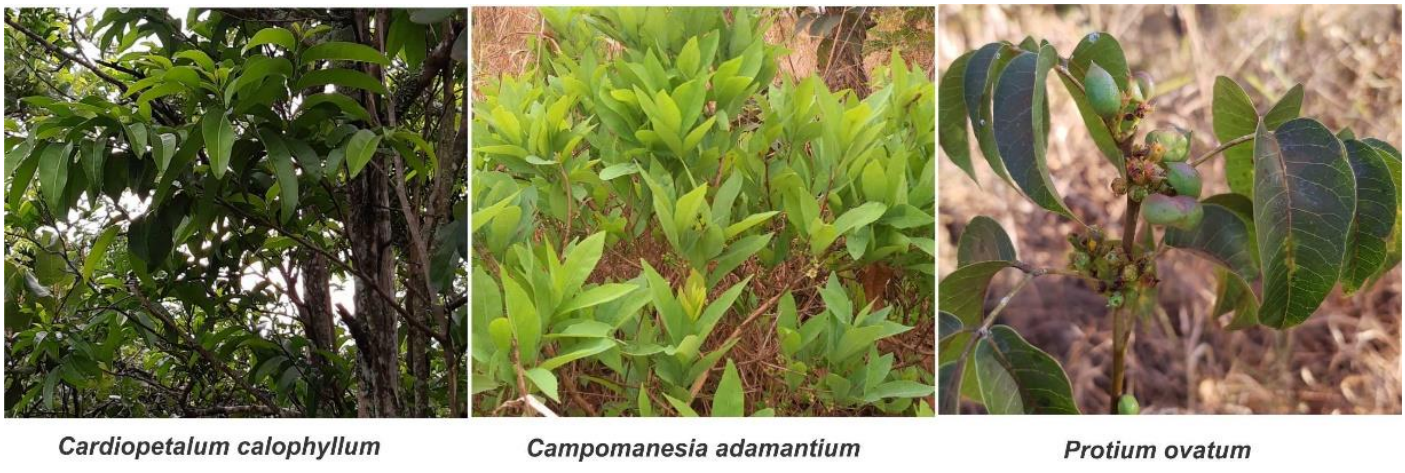

Figure 1. Aerial parts of the three species collected in the *Cerrado* in Goiás state, Brazil

MATERIAL AND METHODS

Plant material

C. adamantium leaves were collected on farms which belong to the Universidade de Rio Verde (UniRV), located in the Rio Verde region, in May 2021. The plant material was deposited at the Herbarium Professor Germano Guarin Neto, in agreement with voucher HJ 6561. *C. calophyllum* leaves were collected in Rio Verde city in March 2014 and the exsiccate was deposited at the herbarium of the Universidade Estadual de Montes Claros (UNIMONTES), in agreement with voucher 3815. *P. ovatum* leaves were collected at the Universidade de Rio Verde (UniRV), located in the *Cerrado* region, in July 2014. The plant was deposited at the Herbarium Jataiense Professor Germano Guarim Neto (exsiccate number HJ 742). All botanic species were identified by the botanist Erika Amaral. Access to the botanical material was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) under the code AEACDCA.

Essential oil extraction

P. ovatum (100 g), *C. adamantium* (100 g) and *C. calophyllum* (100 g) fresh leaves were reduced by a knife mill and had their EOs extracted by hydrodistillation carried out by a Clevenger-type apparatus at 100 °C for 3 h. Afterwards, the hydrolate was subject to liquid-liquid partition in a separatory funnel and washed three times with three 10-mL dichloromethane portions. Samples of EOs were stored at −4 °C for further chemical and biological tests.

Chemical identification of essential oil constituents

EOs were dissolved in ethyl ether and analyzed by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC–MS) with the use of Shimadzu QP5000 Plus and GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems. The temperature of the column in GC-FID was programmed to rise from 60 to 240°C at 3°C/min and was held at 240°C for 5 min; the carrier gas was H_2 at a flow rate of 1.0 mL/min. The equipment was set to operate in the injection mode; the injection volume was 0.1 µL (split ratio of 1:10); and injector and detector temperatures were 240 and 280°C, respectively. Relative concentrations of components were obtained by normalizing peak areas (%). Relative areas consisted of the average of triplicate GC-FID analyses. GC-MS conditions and the identification have been previously reported [11]. Identification of volatile components of EOs (Tables 1-3) was based on their retention indices on an Rtx-5MS (30 m X 0.25 mm; 0.250 µm) capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C_8-C_{20}). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 and their fragmentation patterns were compared with literature data [12].

Anti-*Malassezia furfur* activity of essential oils

In order to evaluate anti-*Malassezia furfur* activity, the chosen methodology is in agreement with procedures described by the literature [14]. The broth microdilution test, with modifications, was carried out as proposed by Leong and coauthors (2017) [15]. A standard strain from the American Type Culture Collection was used: *M. furfur* (ATCC 14521). Concentrations of EOs under investigation ranged from 1.46 to 3000 µg/mL. The culture medium was RPMI-1640 (Gibco), buffered with morpholino propanesulfonic (MOPS, Sigma), pH 7.0, supplemented up to 2% glucose, 1% olive oil (Native), 1% tween 80 (Neon) and 0.5% dry bovine bile (Sigma-Aldrich). Yeast suspension was adjusted by a spectrophotometer (530 nm) in order to reach initial concentration from 0.5 to 2.5 × 10^3 cells/mL. Positive control was ketoconazole (Pfizer), whose concentrations ranged from 0.031 to 16 µg/mL. Both species (strains) *Candida parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were employed as quality controls to monitor the performance of the assay. The 96-well flat-bottom plate was incubated in a microbiological oven at 30ºC for 96 h. In order to reveal yeast growth, 30 µL of 0.01% aqueous solution of resazurin (Sigma) was added to every well [16]. Plates were re-incubated for 24h and then analysis was based on color variations between blue (without any yeast growth) and pink (with yeast growth). Analyses were conducted in triplicate and the lowest concentration of the test sample in which the well remained blue was considered the MIC.

Anti-inflammatory activity of essential oils

The chemotaxis model was used for evaluating anti-inflammatory activity. It was investigated by the Boyden chamber assay [16], as described by Castilhos and coauthors (2007) [17]. This technique enabled the anti-inflammatory potential of EOs from *C. calophyllum*, *C. adamantium* and *P. ovatum* to be evaluated by means of its inhibitory influence on neutrophil motility in suspension (upper compartment) towards the chemotactic agent fMLP 10^{-8} M (lower compartment), determined by the space used by neutrophils through the polycarbonate filter placed between chamber compartments. EOs was dissolved in leukocyte solution at concentrations of 5, 10, 15.7, 24,4 and 32.5 µg/mL and incubated at 37◦C for 30 min. Plasma collected from rats was incubated at 37 ºC for 30 min with 65 µg/mL of LPS (lipopolysaccharide from *Escherichia coli* – the positive control). The plasma was diluted in Hanks buffer at 20% concentration (v/v). An optical microscope (40x magnification) was used for reading the filters. Its focus was initially adjusted to the upper plane of the filter and then slowly deepened to focus on two cells. Reading was carried out in five fields of every filter and the migrating capacity of neutrophils was evaluated in terms of distance, as micrometers, measured between the upper plane of the filter and the final plane of observation [18]. Results showed the mean of distances found by the readings. Results were expressed as mean+/-standard deviation, analyzed by the Student's t-test and compared with the mean of distances determined for non-treated control cells [19]. The difference was considered statistically significant when $p \leq 0.05$. Measurements were taken from five fields across each one of duplicate filters and results were expressed as mean ± standard error of the mean (SEM) and analyzed by one way analysis of variance (ANOVA) followed by the Tukey multiple range test considering $p \leq 0.05$ as significant for chemotactic assay – Tables 5-7.

RESULTS AND DISCUSSION

Volatile constituents of EO-CC, EO-CA and EO-PO fresh leaves were identified by GC-FID and GC–MS. Major compounds were germacrene D (34.9%) and bicyclogermacrene (26.8%) in the EO-CC analysis (Table 1). EO-CA exhibited mainly germacrene D (21.7% - Table 2) while EO-PO exhibited high concentrations of germacrene D (25.0%) and γ-muurolene (18.6%) (Table 3) (Figure 2).

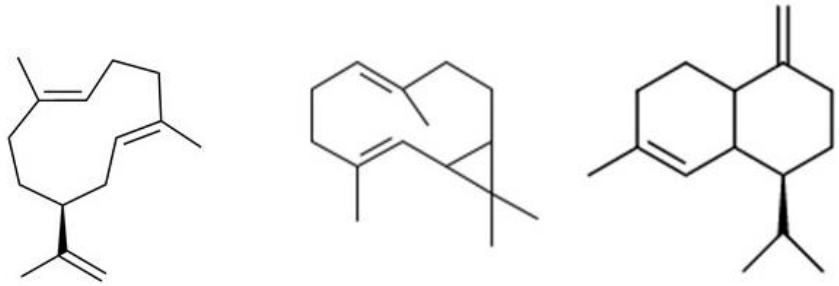

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Figure 2. Chemical structures of major constituents identified in EO-CC, EO-CA and EO-PO: germacrene D (**1**), bicyclogermacrene (**2**) and γ-muurolene (**3**).

Table 1. Volatile constituents of EO-CC

Compound	RT (min)	%RA	RI_{exp}	RI_{lit}
(*E*)-Hexenal	6.35	2.3	853	854
3-Carene	14.80	0.6	1011	1011
δ-Elemene	32.94	3.5	1338	1339
Isocaryophyllene	35.28	7.0	1392	1394
β-Cubebene	35.32	0.5	1389	1390
β-Elemene	35.50	4.0	1393	1391
α-Gurjunene	36.11	0.3	1408	1409
Humulene	37.84	1.7	1453	1455
Germacrene D	38.90	**34.9**	1478	1480
Bicyclogermacrene	39.53	**26.8**	1494	1494

Cont. Table 1

γ-Cadinene	40.15	0.6	1511	1513
α-Cadinene	40.71	1.7	1523	1524
Elemol	41.59	0.8	1548	1549
Germacrene B	41.97	2.8	1560	1561
Spathulenol	42.56	5.1	1573	1575
Globulol	42.67	0.9	1576	1576
Viridiflorol	43.17	0.9	1590	1590
α-Cadinol	44.81	1.8	1652	1653
Total		96.2		

RT = Retention time; **RI_{exp}** = Retention index relative to *n*-alkanes (C_8–C_{20}) on the Rtx-5MS column; **RI_{lit}** = Kovats retention index (values from the literature - [12]). **%RA** = Relative abundance. Bold numbers mean that they were considered major constituents of the EO.

Table 2. Volatile constituents of EO-CA

Compound	RT (min)	%RA	RI_{exp}	RI_{lit}
(*Z*)-Hex-3-enol	6.17	0.5	850	851
α-Pinene	9.46	5.0	938	939
Camphene	10.91	0.7	952	953
β-Pinene	12.68	4.4	980	980
Limonene	15.81	8.6	1031	1031
α-Terpinolene	19.39	0.5	1087	1088
Linalool	19.95	3.2	1096	1098
Fenchyl alcohol	21.05	1.5	1111	1112
Camphene hydrate	23.41	0.4	1146	1148
Borneol	24.51	2.7	1163	1165
4-Terpineol	25.53	0.7	1177	1177
α-Terpineol	26.25	5.2	1187	1189
Bicycloelemene	32.40	2.3	1324	1325
β-Elemene	34.70	2.4	1375	1375
β-Maalien	34.94	0.6	1380	1380
β-Caryophyllene	36.52	7.5	1417	1418
β-Gurjunene	36.71	0.4	1423	1423
Aromadendrene	37.28	3.5	1438	1439
α-Humulene	37.87	2.0	1453	1455
Alloaromadendrene	38.20	2.5	1461	1461
Germacrene D	38.93	**21.7**	1480	1480
Bicyclogermacrene	39.38	7.7	1491	1493
γ-Cadinene	40.13	0.6	1511	1513
δ-Cadinene	40.52	1.9	1523	1524
Germacrene B	42.01	0.1	1560	1560
Ledol	42.31	0.2	1563	1565
Spathulenol	42.63	2.5	1576	1575
epi-Globulol	42.76	3.0	1579	1580
Viridiflorol	42.94	2.2	1588	1590
Rosifoliol	43.48	0.7	1598	1599
γ-Eudesmol	44.53	0.6	1629	1630
Isosphatulenol	44.86	1.3	1639	1639
α-Cadinol	45.38	0.8	1653	1653
Total		97.9		

RT = Retention time; **RI_{exp}** = Retention index relative to *n*-alkanes (C_8–C_{20}) on the Rtx-5MS column; **RI_{lit}** = Kovats retention index (values from the literature - [12]). **%RA** = Relative abundance. Bold numbers mean that they were considered major constituents of the EO.

Table 3. Volatile constituents of EO-PO

Compound	RT (min)	%RA	RI_{exp}	RI_{lit}
3-Hexen-1-ol	6.36	2.5	855	857
β-Pinene	12.80	3.9	979	980
β-Myrcene	13.43	2.1	989	991
3-Carene	14.70	1.5	1009	1011
Limonene	16.01	2.5	1031	1031
cis-β-Ocimene	16.69	1.7	1039	1040
4-Terpineol	25.46	1.2	1176	1177
α-Cubebene	33.51	4.1	1350	1351
β-Elemene	34.71	2.9	1375	1375
β-Caryophyllene	36.49	1.9	1417	1418
Aromadendrene	37.16	2.0	1437	1439
γ-Muurolene	38.71	**18.6**	1475	1477
Germacrene D	38.94	**25.0**	1480	1480
Valencene	39.20	3.2	1489	1490
Bicyclogermacrene	39.38	4.8	1492	1494
α-Bisabolene	39.79	5.2	1503	1504
γ-Cadinene	40.04	2.7	1513	1513
Germacrene B	41.99	5.1	1559	1560
Ledol	42.18	1.8	1564	1565
Viridiflorol	43.12	1.4	1589	1590
τ-Muurolol	44.90	0.3	1639	1640
α-Cadinol	45.31	0.5	1653	1653
α-Bisabolol	46.53	1.4	1682	1683
Total		96.3		

RT = Retention time; RI_{exp} = Retention index relative to *n*-alkanes (C_8–C_{20}) on the Rtx-5MS column; RI_{lit} = Kovats retention index (values from the literature - [12]). **%RA** = Relative abundance. Bold numbers mean that they were considered major constituents of the EO.

The comparison between chemical compositions found by this study and the ones previously reported by the literature shows remarkable differences in terms of concentrations of chemical constituents under identification. Firstly, EO-CC exhibited high concentrations of germacrene D (34.9%) and bicyclogermacrene (26.8%), a fact that differs from the one described by Xavier and coauthors (2016) [3]. They also collected *C. calophyllum* leaves in May and found germacrene D concentration of 4.97% while the major constituent was spathulenol (27.93%) [3]. EO-CA exhibited mostly germacrene D (21.7%) but a previous study of EOs from *C. adamantium* leaves collected in October 2014 and subject to different drying processes showed that germacrene D was not identified by any treatment method [4]. Finally, differences shown by EO-PO should be highlighted. In this study, it exhibited two major constituents, germacrene D (25.0%) and γ-muurolene (18.6%), whereas Estevam and coauthors (2017) [7] reported that they identified the following major constituents in EOs from leaves of this *P. ovatum*: spathulenol (17.6 %), caryophyllene oxide (16.4 %), β-caryophyllene (14.0 %) and myrcene (8.4 %). Its chemical composition was closer to the one described by Sousa and coauthors (2021) [8], who also showed that germacrene D was one of its major constituents.

Several well-known factors, such as temperature, light intensity, ultraviolet radiation, seasonality, water and salinity stress, nutrients and fertilizers in the soil, carbon dioxide concentration, herbivores, pathogens and developmental stages of plants, explain variations in content and chemical composition of EOs [20].

Regarding *in vitro* anti-*Malassezia furfur* activity of EO-CC, EO-CA and EO-PO (Table 4), this pathogen was chosen to be used in tests because it is a lipophilic fungus that causes dermatologic pathologies, such as folliculitis, dermatitis and seborrhea, which are mainly treated with ketoconazole [21]. Ketoconazole was the positive control and exhibited MIC = 0.0625 µg/mL. Both EO-CC and EO-PO exhibited equal values of MIC, i. e., MIC = 750 µg/mL against *M. furfur* while the one of EO-CA was 375 µg/mL. EO-CA was more active than the extract from *P. marginatum* leaves (MIC = 625 µg/mL) [21]. Different EOs have already had

their anti-*Malassezia furfur* activity described by the literature and shown their ethnopharmacological relevance [22]. Donato and coauthors (2020) evaluated antifungal activity of EOs extracted from the following plant genera: *Thymus, Artemisia, Malaleuca, Cinnamomun, Ocimum, Zataria, Rosmarinus, Origanum, Syzigium, Foenicolum, Thapsia, Tachyspermum* and *Myrtus* [22]. EO-CC, EO-CA and EO-PO had close values to the ones of *Salvia rosmarinus* [22]. The literature has determined that samples whose MIC ≤ 500 µg/mL exhibit strong antifungal activity while the ones whose MIC ≤ 1000 µg/mL have moderate antifungal activity [23]. For this reason, EO-CA was considered highly active against *M. furfur* while EO-CC and EO-PO were moderately active. The presence of the major constituent – germacrene D – of the three EOs under study is noteworthy, since this sesquiterpene has been known in the current literature due to its high antibacterial and antifungal activities [28].

Studies have shown that some EOs exhibit antifungal activity due to the fact that the lipophilic nature of their terpenoid components may rupture membranes of fungal cells and cause their death. Monoterpenes bind to ergosterol and lead to destabilization of fungal cell membranes due to inhibition of ergosterol biosynthesis and block yeast growth, such as *M. furfur* growth. By inhibiting their growth in S phase of the cell cycle, EOs interfere with the morphogenetic signaling pathway of pseudohyphae, thus, preventing formation of resistant biofilms [23].

Firstly, the reason why the chemotaxis model was chosen to evaluate anti-inflammatory activity should be explained. Concerning cells with high capacity to respond to chemotactic stimuli, circulating granulocytes in peripheral blood have stood out. Neutrophils, which are also known as polymorphonuclear (PMN) neutrophils, comprise the primary cell population in defense against several types of microorganisms found in the environment since they quickly accumulate in invasive sites or lesions. Their participation in inflammatory processes is multifunctional and involves certain steps: the first is to selectively recognize the aggressor, the second is to respond appropriately by locomotion, the third is microorganism phagocytosis and the last one is elimination [24, 25]. Therefore, they are capable of secreting substances that can not only retard infection dissemination but also recruit other types of leucocytes to move to infectious/inflammatory sites whenever necessary. Their polylobulated nuclei enable to cross pores that are much smaller than their diameters, a feature that works as a requirement to their ability to compact, to go through the endothelial pavement (diapedesis) and to keep moving towards inflammatory sites by chemotaxis [24, 25].

Neutrophils are cells that participate in innate defense; in tissues, they can be found in all sites of entries in the human body that are in contact with the external environment. Chemotaxis, i. e., cell movement towards (positive chemotaxis) or against (negative chemotaxis) a chemical gradient, is common in several eukaryotic cells; in the case of leukocytes, it is the mechanism they use to accumulate in inflammatory sites [24, 25].

Exposure of human leukocytes to different concentrations of all EOs (EO-CC, EO-CA and EO-PO) resulted in direct dose-dependent inhibition of PMN at the following doses: 5, 10, 15.7, 24.4 and 32.5 µg/mL (Tables 5-7). Results show that EOs under evaluation are capable of interfering with PMN chemotaxis, which was investigated in a specific *in vitro* system to evaluate this function. Therefore, anti-inflammatory activity may be related to its capacity to change mechanisms that involve recruitment of cells engaged in inflammatory response. In EO-CC, the highest inhibition value – 94% – was found at concentration of 24.4 µg/mL, since, at the highest concentration under investigation, i. e., 32.5 µg/mL, there was no change in the percentage (Table 5). EO-CA showed 96% of inhibitory potential at doses of 15.7, 24.4 and 32.5 µg/mL (Table 6). The evaluation of EO-PO showed the best and highest inhibition percentage, i. e., 97%, at concentration of 32.5 µg/mL (Table 7).

Inhibitory effects of medicinal plants on PMN chemotaxis, such as the ones described by this study, have also been related to EOs from other plants [26]. For instance, EOs from *Hypericum perforatum* and some of their pure chemical constituents were also tested separately [27]. Schepetkin and coauthors (2020) evaluated anti-inflammatory activity of germacrene D and found IC_{50} = 5.4 µg/mL [27]. Based on this recently published data, the authors of the study reported by this paper suggest that good anti-inflammatory activity exhibited by EO-CC, EO-CA and EO-PO is mostly due to germacrene D, besides the synergic effect of other constituents found at lower concentrations [29-30].

Even though EOs evaluated by this study showed suggestive results of their participation in chemotaxis, the possibility of their involvement in other intracellular mechanisms that participate in the inflammatory response cannot be excluded. In-depth studies of these effects may be carried out by specific experimental assays to investigate cell mechanisms at molecular levels.

Table 4. *In vitro* antifungal activities of EO-CC, EO-CA and EO-PO (MIC = µg/mL)

Samples	*M. furfur*
EO-CC	750
EO-CA	375
EO-PO	750
Ketoconazole*	0.0625

*Positive control

Table 5. *In vitro* chemotactic response of neutrophils treated with the suspension of EO-CC

Concentration (µg/mL)	Distance migrated (µm)	% Inhibition
5	10 ± 1	70
10	7 ± 1	75
15.7	8 ± 2	89
24.4	8 ± 2	94
32.5	9 ± 2	94
Control*	113 ± 1	100

Results of distance migrated are mean ± standard error of the mean. *Positive control: lipopolysaccharide from *Escherichia coli*.

Table 6. *In vitro* chemotactic response of neutrophils treated with the suspension of EO-CA

Concentration (µg/mL)	Distance migrated (µm)	% Inhibition
5	5 ± 2	72
10	6 ± 1	84
15.7	8 ± 2	96
24.4	8 ± 2	96
32.5	8 ± 2	96
Control*	113 ± 1	100

Results of distance migrated are mean ± standard error of the mean. *Positive control: lipopolysaccharide from *Escherichia coli*.

Table 7. *In vitro* chemotactic response of neutrophils treated with the suspension of EO-PO.

Concentration (µg/mL)	Distance migrated (µm)	% Inhibition
5	6 ± 1	81
10	7 ± 1	87
15.7	8 ± 1	95
24.4	9 ± 1	96
32.5	10 ± 1	97
Control*	113 ± 1	100

Results of distance migrated are mean ± standard error of the mean. *Positive control: lipopolysaccharide from *Escherichia coli*.

CONCLUSION

Results of this study highlight OEs of three species found in the *Cerrado* in GO, Brazil, as promising alternatives to develop new antifungal and anti-inflammatory agents for clinical use. EO-CA was the one that better exhibited antifungal activity against *M. furfur*. Regarding anti-inflammatory activity, EO-PO was the most active one in *in vitro assays*. In sum, this study is an original and important contribution to chemical and pharmacological knowledge about *C. calophyllum*, *C. adamantium* and *P. ovatum.*

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