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Composição Química e Atividade Acaricida contra *Tetranychus urticae* do Óleo Essencial da Planta *Marsypianthes chamaedrys* (Vahl.) Kuntze

Resumo: *Marsypianthes chamaedrys (Vahl.) Kuntze* é uma planta popularmente conhecida como paracari, erva de cobra, bóia-caá ou betônia brava e distribuída principalmente nas regiões Norte e Nordeste do Brasil. As partes aéreas da *Marsypianthes chamaedrys* (folhas e caule) foram utilizadas para extração do óleo essencial, sendo os constituintes identificados por análises de CG-MS e a atividade acaracida do óleo foi avaliada. As análises por GC-MS identificaram 29 compostos, sendo identificados os sesquiterpenos β-cariofileno (12,2%), biciclogermacreno (17,9%) e germacreno D (34,1%). O óleo essencial obtido foi avaliado contra *Tetranychus urticae* mostrando nenhuma atividade acaricida significativa.

Palavras-chave: *Marsypianthes chamaedrys*; *Tetranychus urticae*; sesquiterpeno; GC-MS.

Abstract

Marsypianthes chamaedrys (Vahl.) Kuntze, known as paracari, erva de cobra, bóia-caá or betônia brava, is a common herb in Brazil (North and Northeast regions). The aerial parts (leaves and stem) of this plant were used to essential oil extraction. The essential oil obtained was evaluated against *Tetranychus urticae* showing no significant acaricidal activity. Analysis by GC-MS was performed and 29 compounds were identified and the main compounds were the sesquiterpenes β-caryophyllene (12.2%), bicyclogermacrene (17.9%) and germacrene D (34.1%).

Keywords: *Marsypianthes chamaedrys*; *Tetranychus urticae*; sesquiterpene; GC-MS.

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Chemical Composition and Acaricidal Activity against *Tetranychus urticae* of Essential Oil from *Marsypianthes chamaedrys* (Vahl.) Kuntze

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1. Introduction

Plants of the genus *Marsypianthes* belong to the family Lamiaceae and occur more frequently in the North and Northeast of Brazil, where some species are popularly known as paracari, erva de cobra, bóia-caá or betônia brava.[1]This specie is used in the folk medicine as anti-snake venom.[2] The extract of *Marsypianthes chamaedrys* (Vahl.) Kuntze presented anti-ophidian activity, protecting mice against the venom of *Bothrops jararaca.*[1,2] Moreover, studies reported the analgesic effect of *M. chamaedrys* leaf extract, measured by the number of contractions and anti-inflammatory effect, as assessed by intraperitoneal dissemination of Evans blue.[1,3] Previous chemical investigations led to the isolation and identification of steroids β-sitosterol and stigmasterol, triterpenes ursolic acid, oleanoic acid and tormentic acid from the aerial parts of *M. chamaedrys*. Other studies showed that this species is rich in triterpenes and sesquiterpenes, especially the oleanolic, ursolic and tormentic acids, lupeol, germanicol, α-amyrin and β-amyrin, β-caryophyllene, germacrene D and presents flavonoids, such as rutin.[4-6]

A recent review has reported the effectiveness of essential oils on repellency of insects that transmit neglected diseases and a detailed analysis shows the great number of patents on this subject.[7] Furthermore, chemical substances obtained from plant species are one of the more efficient alternatives for pest control.[8] Many studies led to the identification of several natural compounds from plant species with insecticide and/or acaricidal activity[9] and then are pyrethrum (extracted from chrysanthemum - *Chrysanthemum cinerariaefolium Trev*.), nicotine (from *Nicotiana tabacum L*.), rotenone (from *Derris sp.* and *Lonchocarpus sp.*)and azadirachtin (from *Azadirachta indica A. Juss*.), widely used today.[10] In this way, it is necessary to investigate the possible toxicity of essential oils from different plants on insects and mites.[11]

The two-spotted spider mite, *Tetranychus urticae*, are important mite species of agriculture. *T. urticae* is able to infest a wide variety of economically important crops in the world[12], such as cotton, bean, strawberry, papaya, tomato and grape[13], causing considerable losses in productivity.[14]This mite has an oval body and displays marked sexual dimorphism, with females measuring about 0.5 mm and males to 0.25 mm male. The dorsal shield are covered by long arrows and have two dark spots, one located on each side of the back.[15] In the colonization of plants, females weave silk, which acquire the form of a web, where they form large colonies. The eggs are easily visible to the naked eye and are preferably placed on the lower leaf surface. Development phases are egg, larva, with three pairs of legs, protonymph, deutonymph and adult with four pairs of legs[16]. Due the high damage potential of *T. urticae* on crops and the need to develop control methods less harmful to the environment, this study aimed to determine the chemical composition of essential oil obtained from *Marsypianthes chamaedrys* and check its possible fumigant effect on two-spotted spider mites.

2. Experimental

2.1. Plant material

The aerial parts of *Marsypianthes chamaedrys* were collected at Passos (Minas Gerais, Brazil) and identified by. Prof. Dr. Milton Groppo Junior. A voucher specimen (N° SPFR 12160, collector number: Milton Groppo 1887) was deposited at the herbarium (SPFR) of the Biology Department, Faculdade de Filosofia Ciências e Letras of Ribeirão Preto of São Paulo University, Ribeirão Preto, SP, Brazil. The field work and the chemical investigation were done with authorization of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA (authorization number

26625-1).

2.2. Essential oil isolation

The fresh aerial parts (leaves and stem) of *M. chamaedrys* (1.07 Kg) were extracted by hydrodistillation using Clevenger-type apparatus for 4h. The oil (67.6 mg) was dried at anhydrous sodium sulphate and was analyzed by GC-MS.

2.3. GC-MS analysis

GC-MS analysis was done on a Shimadzu QP2010 with AOC-20i auto-injector using DB-5MS column (30 m x 0.25 mm, 0.25 mm in thickness). The carrier gas was He with pressure of 57.4 kPa and flow rate of 1.00 mL/min. The split ratio was 1/30, the injector temperature was 250˚C and the injected volume was 1μL. Temperature programming was the following: 60 – 240˚C increasing in 3˚C/min. MS were recorded on electron ionization (EI) mode, with ionization energy of 70 eV (scan time: 2 scans/s). The identification of constituents was carried out based on the retention indices (calculated using from C9 to C22 alkanes) and by comparing the mass spectra with a computer databank (WILEY 7 and NIST 62) and with reference to published data.[17]

2.4. Biological material

Tetranychus urticae were obtained from cultures maintained for 10 years without exposure to any acaricide in the Plant Protection Department, São Paulo University. Spider mites were reared on common bean plants (3-5week-old), *Phaseolus vulgaris* L., variety Cerinza - IAC, at 25±2°C and 65±5%r.h. and L12: D12. Tests were also carried out under the same conditions. Adult's forms of *Tetranychus urticae* were treated with samples *M. chamaedrys* oil.

2.5. Bioassays

Desiccators with a capacity of 0.5 L were used as test chambers. Common bean leaves (3 wk after germination) were collected and disks (3 cm in diameter) were punched from each leaf. Four leaf disks with adult *T. urticae* (2-3 d old) were placed on water-soaked cotton pads at the bottom of the desiccators. Each replicate consisted of 10 *T. urticae* adults placed on different leaf disks. A fine brush was used to transfer the spider mites onto leaves. For each dose and exposure time combination, four replicates were used in a completely randomized design. The essential oils were applied with an automatic pipette on a blotting paper strip (6 cm × 3 cm) attached to the bottom of the desiccators.

The amounts of essential oils applied were 1, 2, 3 and 4 µL/L air. No material was applied to the control desiccators. Exposure periods were 24 and 48 h to determine mortalities during each exposure time, leaf disks with two-spotted spider mites were removed from the desiccators and incited with a fine brush, if mites did not move, they were considered to be dead.

2.6. Data Analysis

In order to determine if there is a statistically significant difference in toxicity among the acaricidal doses in two exposure times, data were analyzed with a one-way analysis of variance (ANOVA); for analysis, the original data (x) were transformed to arcsen $((x + 0.5)^{1/2}/100)$. The means were compared using the Tukey test.[18] Statistical analyses were performed using the PROC GLM procedure in the SAS software package.[19]

3. Results and Discussion

The essential oil of aerial parts from *M. chamaedrys* was obtained and analyzed by GC-MS to determine its composition. Moreover, this oil was evaluated against *T. urticae* to establish a possible fumigant effect. Twenty nine compounds were detected and 26 of them were identified (Table I, Figure 1). The main identified compounds were β-caryophyllene (12.2 %), bicyclogermacrene (17.9 %), germacrene D (34.1 %), δ-elemene (3.8 %) and β-elemene (3.0 %). The sesquiterpene β-caryophyllene has shown anti-inflammatory[20], local anesthetic [21] and antimicrobial activities.[22] Germacrene D was already identified in various plants and probably it plays a role in the signposting of plants.[23] In addition germacrene D is reported to be a biosynthetic precursor of the other sesquiterpenes, such as cadinanes, muurolanes and amorphanes.[24]

Some monoterpenes were observed such as β-myrcene (0.2 %), limonene (0.6 %) and (*Z*)-β-ocimene (0.5 %). In addition, oxygenated sesquiterpenes were also identified: ledol (0.3 %), spathulenol (1.7 %), caryophyllene oxide (2.0 %), globulol (1.8 %), viridiflorol (0.6 %), iso-spathulenol (2.0 %), *epi*-α-cadinol (1.9 %), tau-muurolol (0.6 %) and α-cadinol (2.2 %).

Table 1. Compounds identified in essential oil of *Marsypianthes chamaedrys* by GC-MS

Peak	RT (min)	Compound	% relative	RI
1	7,28	β-myrcene^{M}	0,20	987
2	8,66	Limonene^{M}	0,61	1028
3	9,24	(*Z*)-β-ocimene^{M}	0,52	1042
4	21,16	Bicycloelemene^{S}	0,68	1328[1]
5	21,30	δ-elemene^{S}	3,85	1332
6	23,02	α-copaene^{S}	1,94	1372
7	23,61	β-elemene^{S}	3,01	1386
8	24,84	β-caryophyllene^{S}	12,21	1415
9	25,27	β-gurjunene^{S}	0,32	1426
10	25,44	(*E*)-α-bergamotene^{S}	0,33	1430
11	26,32	α-humulene^{S}	2,81	1451
12	27,39	germacreneD^{S}	34,10	1476
13	27,97	bicyclogermacrene^{S}	17,88	1490
14	28,42	Germacrene A^{S}	1,11	1501
15	28,68	γ-cadinene^{S}	0,72	1508
16	28,78	NI	0,44	1510
17	28,89	δ-cadinene^{S}	1,68	1514
18	29,44	NI	2,67	1527
19	30,45	GermacreneB^{S}	1,24	1553
20	30,63	Ledol^{S}	0,32	1559
21	31,16	Spathulenol^{S}	1,72	1571
22	31,35	Caryophyllene oxide^{S}	2,05	1576
23	31,51	Globulol^{S}	1,80	1580
24	31,85	Viridiflorol^{S}	0,59	1588
25	33,07	NI	0,59	1620
26	33,15	Isospathulenol^{S}	1,97	1624
27	33,69	*epi*-α-cadinol^{S}	1,87	1636
28	33,76	Tau-muurolol^{S}	0,58	1638
29	34,18	α-cadinol^{S}	2,19	1650

RT: retention time; ^{M}monoterpenE; ^{S}sesquiterpene RI: retention index in DB-5MS according to Ferhat *et al.*, 2007; [25]. Javidnia *et al.*, 2007; [26]Pino *et al.*, 2005[27]

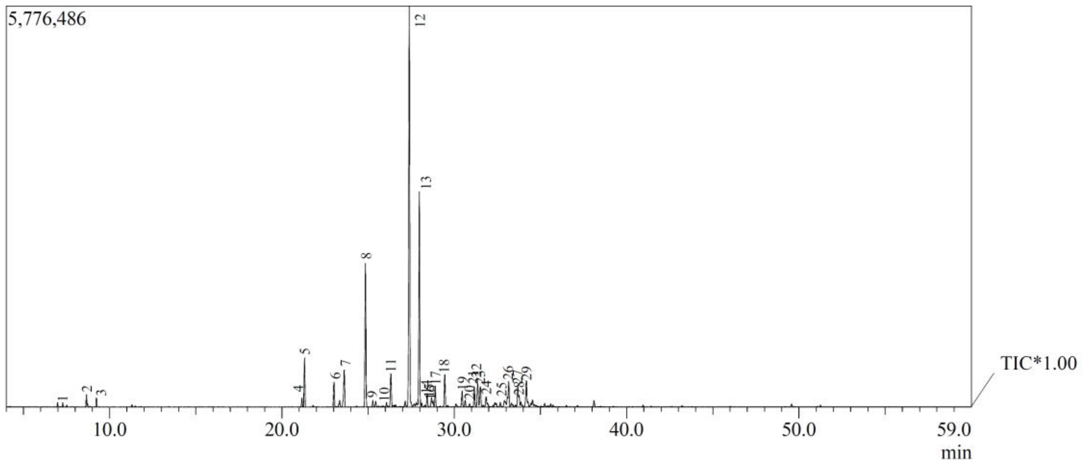

Figure 1. GC-MS chromatogram of the essential oil from aerial parts (leaves and stem) of *Marsypianthes chamaedrys*

The acaricidal effects of essential oil obtained from *M. chamaedrys* were summarized in Figure 2. The results showed that essential oil do not affect adults of *T. urticae*. There were no significant differences (*P>0.05*) between the mortality levels in terms of the essential oil concentrations regardless of exposure time. The results of this work suggest low acaricidal activity at the doses evaluated. The concentrations used in our assays (1-5 µL/L) were similar to those used in other similar studies (1,4-9,3 µL/L), confirming the low efficiency of this oil.[28-30] The highest mortality rate was 15% (2 µL/L) with greatest exposure time (48h).

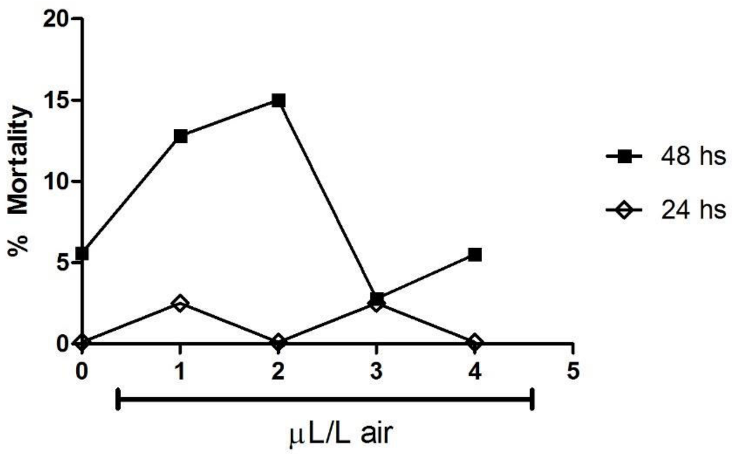

Figure 2. Mortality (%) of *Tetranychus urticae*at different doses after 24 h and 48 of exposure

Probably, dose increasing may improve the efficiency of control to two-spotted spider mite, but this complicates the use of this plant species as natural pesticide, since it requires the collection and processing a large volume of plant parts. It is important to highlight that the fractions and triterpenes mixtures of *M. chamaedrys* had showed prominent molluscicidal activity.[28]

The essential oil was not efficient against *T. urticae*, however it is important to evaluate other biological activities, because many activities have been reported for germacrene D and β-caryophyllene. Moreover, the essential oil of *M. chamaedrys* could be used as a source of germacrene D.

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References

[1] Ruppelt, B. M.; Pereira, E. F. R.; Gonçalves, L. C.; Pereira, N. A. Pharmacological screening of plants recommended by folk medicine as anti-snake venom-I. Analgesic and anti-inflammatory activities. *Memórias do Instituto Oswaldo Cruz* **1991**, *86*, 203. [CrossRef] [PubMed]

[2] Castro, K. N.; Carvalho, A. L.; Almeida, A. P.; Oliveira, D. B.; Borba, H. R.; Costa, S. S.; Zingali, R. B. Preliminary in vitro studies on the *Marsypiantheschamaedrys* (boia-caá) extracts at fibrinoclotting induced by snake venoms. *Toxicon* **2003**, *41*, 929. [CrossRef] [PubMed]

[3] Magalhães, A.; Santos, G. B.; Verdam, M. C.; Fraporti, L.; Malheiro, A.; Lima, E. S.; Santos, M. C. Inhibition of the inflammatory and coagulant action of bothrops atrox venom by the plant species *Marsypianthes chamaedrys*. *Journal of Ethnopharmacology* **2011**, *8*, 82. [CrossRef] [PubMed]

[4] Menezes, F. S.; Borsatto, A. S.; Pereira, N. A.; Matos, F. J. A.; Kaplan, M. A. C. Chamaedrydiol, an ursane triterpene from *Marsypianthes chamaedrys*. *Phytochemistry* **1998**, *48*, 323. [CrossRef]

[5] Matos, F. J. A; Machado, M. I. L.; Craveiro, A. A.; Alencar, J. W.; Meneses, F. S. Essential Oil Composition of *Marsypianthes chamaedrys* (Vahl) Kuntze Grown in Northeast Brazil. *Journal of Essential Oil Research* **2001**, *3*, 45. [CrossRef]

[6] Hashimoto, M. Y.; Costa, D. P.; Faria, M. T.; Ferreira, H. D.; Santos, S. C.; Paula, J. R.; Seraphind, J. C.; Ferri, P. H. Chemotaxonomy of *Marsypianthes* Mart. ex Benth. Based on Essential Oil Variability. *Journal of the Brazilian Chemical Society* **2014**, *25*, 1504. [CrossRef]

[7] Pohlit, A. M.; Lopes, N. P.; Gama, R. A.; Tadei, W. P.; Neto, V. F. Patent literature on mosquito repellent inventions which contain plant essential oils - a review. *Planta Medica* **2011**, *77*, 598. [CrossRef] [PubMed]

[8] Castiglioni, E.; Vendramin, J. D.; Tamai,M. A. Evaluación del efecto de extractos acuosos y derivados de meliáceas sobre *Teatranychus urticae* (Koch) (*Acari, Tetranychidae*). *Agrociência* **2002**, *6*, 75. [CrossRef]

[9] Viegas Junior, C. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Química Nova* **2003**, *26*, 390. [CrossRef]

[10] Roel, A. R. Utilização de plantas com propriedades inseticidas: uma contribuição para o desenvolvimento rural sustentável. *Revista Interncional de Desenvolvimento Local* **2001**, *1*, 43. [Link]

[11] Tunç, I.; Fahinkaya, S. Sensitivity of two greenhouse pests to vapours of essential oils. *Entomologia Experimentalis et Applicata* **1998**, *86*, 183. [CrossRef]

[12] Robinson-Vargas, M.; Chapman, B.; Penman, D. R. Toxicity of thuringiensin on immature and adult stages of *Tetranychus urticae* Koch and *Panonychusulmi* (Koch) (*Acari, Tetranychidae*). *Agricultura Técnica* **2001**, *61*, 3. [CrossRef]

[13] Gallo, D.; Nakano, O.; Silveira-Neto, S.; Carvalho, R. P. L.; Batista, G. C.; Berti-Filho, E.; Parra, J. R. P.; Zucchi, R. A.; Alves, S. B.; Vendramin, J. D.; Marchini, L. C.; Lopes, J. R. S.; Omoto, C. Entomologia Agrícola 2002, Piracicaba, FEALQ, 920p. [Link]

[14] Cloyd, R. A.; Galle, C. L.; Keith, S. R.; Kalscheur, N. A.; Kemp, K. E. Effect of commercially available plant-derived essential oil products on arthropod pests. *Journal of Economic Entomology* **2009**, *102*, 1567. [CrossRef] [PubMed]

[15] Fadini, M. A. M.; Pallini, A.; Venzon, M. Controle de ácaros em sistema de produção integrada de morango. *Ciência Rural*, **2004**, *34*, 1271. [CrossRef]

[16] Moraes, G.; Flechtmann, C. H. W.; *Manual de acarologia – acarologia básica e ácaros de plantas cultivadas no Brasil,* 1a. ed., Holos: Ribeirão Preto, 2008.

[17] Adams, R. P.; *Identification oil components by gas chromatography/mass spectroscopy*, 4a. ed., Allured Publ. Corp.: Carol Stream, IL, 1995.

[18] Winer, B. J.; Brown, D. R.; Michels, K. M. *Statistical principles in experimental design*, 3ed., McGraw-Hill: New York, USA, 1991**.**

[19] SAS Institute – *Sas/stat user's guide, version 8.1*. SAS Institute, Cary (2001).

[20] Fernandes, E. S.; Passos, G. F.; Medeiros, R.; Cunha, F. M.; Ferreira, J.; Campos, M. M.; Pianowski L. F.; Calixto, J. B. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordiaverbenacea. European Journal of Pharmacology* **2007***, 569*, 228. [CrossRef] [PubMed]

[21] Ghelardini, C.; Galeotti, N.; Mannellia, L. D. C.; Mazzanti, G.; Bartolini. A. Local anesthetic activity of β-caryophyllene. *Farmaco* **2001**, *56*, 387. [CrossRef]

[22] Sabulal, B.; Dan, M.; John, A.; Kurup, A.; Pradeep, N. S.; Valsamma, R. K.; George, V. Caryophyllene-rich rhizome oil of *Zingibernimmonii* from South India: Chemical characterization and antimicrobial activity. *Phytochemistry* **2006**, *67*, 2469. [CrossRef] [PubMed]

[23] Mozuraitis, R.; Straden, M.; Ramirez, M. I.; Borg-Karlson, K.; Mustaparta, H. Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chemical Senses* **2002**, *27*, 505. [CrossRef] [PubMed]

[24] Bülow, N.; König, W. A. The role of germacrene D as a precursor in sesquiterpene biosynthesis: investigations of acid catalyzed, photochemically and thermally induced rearrangements. *Phytochemistry* **2000**, *55*, 141. [CrossRef]

[25] Ferhat, M. A.; Tigrine-Kordjani, N.; Chemat, S.; Meklati, B. Y.; Chemat, F. Rapid extraction of volatile compounds using a new simultaneous microwave distillation: solvent extraction device. *Chromatographia* **2007**, *65*, 217. [CrossRef]

[26] Javidnia, K.; Nasiri, A.; Miri, R.; Jamalian, A. Composition of the essential oil of *Helianthemum kahiricum* Del. from Iran. *Journal of Essential Oil Research* **2007**, *19*, 52. [CrossRef]

[27] Pino, J. A.; Mesa, J.; Munoz, Y.; Martiä, M. P.; Marbot, R. Volatile componentes from Mango (*Mangifera indica* L.) cultivars. *Journal Agricultutal and Food Chemistry* **2005**, *53*, 2213. [CrossRef] [PubMed]

[28] Menezes, F. S.; Silva, C. S.; Pereira, N. A.; Matos, F. J. A.; Borsatto, A. S. B; Kaplan, M. A. C. Molluscicidal constituents of *Marsypianthes chamaedrys*. *Phytotherapy Research* **1999**, *13*, 433. [CrossRef] [PubMed]

[29] 29. Choi, W.; Lee, S. G.; Park, H. M.; Ahn, Y. J. Toxicity of Plant Essential Oils to *Tetranychusurticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Economic Entomology* **2004**, *97*, 553. [CrossRef] [PubMed]

[30] 30. Çalmasur, Ö.; Aslan, I.; Sahin, F. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisiatabaci* Genn. *Industrial Crops and Products* **2006**, *23*, 140. [CrossRef]