Chemical composition and antioxidant activity of Tagetes filifolia Lag. essential oil from Peruvian Andes

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Composición química y actividad antioxidante de Tagetes filifolia Lag. aceite esencial de los andes peruanos

Composició química i activitat antioxidant de Tagetes filifolia Lag. oli essencial dels Andes peruans

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ABSTRACT

The chemical composition of the essential oil from the aerial parts (leaves and flowers) of *Tagetes filifolia* Lag. grown in the Peruvian Andes was examined throughout the gas chromatography and gas chromatography-mass spectrometry techniques. Fifty-seven compounds were identified representing more than 99% of the essential oil. Phenylpropanoids had the major percentage, including (*E*)-anethol (85.0%) and estragole (12.9%) as the most abundant compounds. A multivariate analysis was performed using hierarchical cluster analysis to examine the relationship between the studied sample and other *T. filifolia* specimens from 21 essential oil compositions reported in the literature. Agglomerative hierarchical cluster analysis reveals three groups: (*E*)-anethole/estragole, estragole/(*E*)-anethole and an outlier (*Z*)-anethole/estragole. In addition, antioxidant activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl and ferric reducing antioxidant power assays. The essential oil had a moderate scavenging effect and ferric reducing capacity.

Key words: *Tagetes filifolia*, essential oil, chemical composition, hierarchical cluster analysis, antioxidant activity.

RESUMEN

La composición química del aceite esencial de las partes aéreas (hojas y flores) de *Tagetes filifolia* Lag. cosechado en los Andes peruanos se examinó mediante las técnicas de cromatografía de gases y cromatografía de gases-espectrometría de masas. Se identificaron cincuenta y siete compuestos que representan más del 99% del aceite esencial. Los fenilpropanoides tuvieron el mayor porcentaje, incluyendo (*E*)-anetol (85,0%) y estragol (12,9%) como los compuestos más abundantes. Se realizó un análisis multivariado mediante análisis de conglomerados jerárquico para examinar la relación entre la muestra estudiada y otros especímenes de *T. filifolia* de 21 composiciones de aceites esenciales reportadas en la literatura. El análisis de conglomerados jerárquicos revela tres grupos: (*E*)-anetol/estragol, estragol/(*E*)-anetol y un valor atípico (*Z*)-anetol/estragol. Además, la actividad antioxidante se evaluó mediante ensayos de 1,1-difenil-2-picril-hidrazilo y de poder antioxidante reductor férrico. El aceite esencial tuvo un moderado efecto depurador y capacidad reductora de hierro.

Palabras clave: *Tagetes filifolia*, aceite esencial, composición química, análisis de conglomerados jerárquicos, actividad antioxidante.

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RESUM

La composició química de l'oli essencial de les parts aèries (fulles i flors) de *Tagetes filifolia* Lag. collit als Andes peruans es va examinar mitjançant les tècniques de cromatografia de gasos i cromatografia de gasos-espectrometria de masses. S'hi van identificar cinquanta-set compostos que representen més del 99% de l'oli essencial. Els fenilpropanoides van tenir el percentatge més gran, incloent (*E*)-anetol (85,0%) i estragol (12,9%) com els compostos més abundants. Es va realitzar una anàlisi multivariada mitjançant anàlisis de conglomerats jeràrquics per examinar la relació entre la mostra estudiada i altres espècimens de *T. filifolia* de 21 composicions d'olis essencials reportades a la literatura. L'anàlisi de conglomerats jeràrquics revela tres grups: (*E*)-anetol/estragol, estragol/(*E*)-anetol i un valor atípic (*Z*)-anetol/estragol. A més, l'activitat antioxidant es va avaluar mitjançant assaigs d'1,1-difenil-2-picril-hidrazil i de poder antioxidant reductor fèrric. L'oli essencial va tenir un efecte depurador moderat i capacitat reductora de ferro.

Paraules clau: *Tagetes filifolia*, oli essencial, composició química, anàlisi de conglomerats jeràrquics, activitat antioxidant.

INTRODUCTION

Tagetes spp. is an important genus belonging to the Asteraceae family and is native to America, but it is naturalized in many regions of Africa, Asia and Europe[1]. The species are cultivated as ornamental plants or are found as wild and many of the extracts or essential oils are studied for their biological activities [2-5]. In fact, diverse *Tagetes* spp. are used in the folk medicine for the treatment of different illness [1].

Tagetes filifolia Lag. is an aromatic herb and its cultivation extending from Mexico to Argentina; in the Peru it is in Amazons, Ancash, Cajamarca, Cusco, Huánuco, The Freedom and Lambayeque [6]. There are reports in Latin American countries about the use of infusions of the aerial parts as diuretic, antispasmodic, tonic, anti-inflammatory, antihemorrhagic, emmenagogue, antifungal, anthelmintic and food flavoring [7-11]. The plant is locally named as `pampa anis', `anis k'ita', `anis-anis', `anis silvestre' and `tuna anicillo' in Perú [12] and 'anisillo' in Mexico and Argentina [13]. The essential oil from leaves and flowers are recognized as fungicide, insecticide, nematicide and repellent [13-19]. The antioxidant activity of the essential oil was evaluated only in one study and determined only by the DPPH test [18]. Many chemotypes and variants are grown all over the world. The accessions from different climatic variations at altitudes ranged 750-3500 m presented a specific composition of estragole or *p*-allylanisole and of (*E*)-anethole as only major compound or a mixture of both phenylpropanoid compounds from plats grown in Argentine [13, 14, 21, 22, 28, 31, 33], Mexico [20, 26, 27], Peru [23], Costa Rica [24], Italy [25], Ecuador [29, 30], and Venezuela [32]. Only in one study, (*Z*)-anethole was reported as the major component of the essential oil obtained from plants grown at 3500 m[23].

Based on the abovementioned information, the aims of the present study were to evaluate the composition of the essential oil and its antioxidant activity from aerial parts (leaves and flowers) of *Tagetes filifolia* Lag. from the Andean region of Peru at 3953 m above sea level.

MATERIALS AND METHODS

Plant material

Leaves and flowers of *T. filifolia* were collected from flowering plants growing wild in Occopa, district of Santiago, in Cuzco, Peru (3953 m above sea level) during September 2019. Three repetitions at the same location were sampled with 10 plants in each group. Authentication was performed by botanists of the Herbarium Vargas at the Biological Sciences Faculty of the Universidad Nacional de San Antonio Abad del Cusco, and a voucher specimen was deposited at the herbarium (accession number 45713CUZ).

Isolation of the essential oil

Three samples (200 g) from fresh different plants were hydrodistilled for 3 h in a Clevenger-type apparatus with total immersion of the biomass. Essential oils were dried over anhydrous sodium sulfate and stored at -5°C until analysis.

Chromatographic analysis

The essential oil was analyzed by GC-FID using a Hewlett-Packard 6890N series II GC (Agilent, Santa Clara, CA, USA) equipped with a DB-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) or DB-Wax column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA). The temperature program for both columns was 70°C (after 2 min) to 250°C at 4°C/min and held for 10 min using hydrogen as carrier gas at 1 mL/min. Injector and flame ionization detector temperatures were 250°C and injection size was 0.1 mL prepared at 10 % v/v in diethyl ether; split ratio 1:20. Linear retention indexes were calculated using a mixture of *n*-alkanes (C_8-C_{24}). Quantification of compounds was carried out using relative percentage abundance and normalization method with correction response factors based on grouping the essential oil components by their functional groups [34]. Percentage data are the mean values of two injections per sample.

The essential oil was also examined by GC-MS using a Trace 1310 GC-MS (Thermo Scientific, Waltham, Massachusetts, USA) fitted with a 30 m x 0.25 mm i.d. x 0.25 µm DB-5MS column. The temperature program and injection mode were the same as in GC-FID. Helium as carrier gas at a flow rate of 1 mL/min; electron ionization system with ionization energy of 70 eV was used; mass range: 35-400 m/z; injector, ion source and connecting parts temperature were at 250°C. Compounds were identified using their linear retention indices (RI) calculated by linear interpolation relative to *n*-alkanes and mass spectra. Linear retention indices were compared against corresponding reference standards and those found in NIST Standard Reference Database (https://webbook.nist.gov/chemistry). Mass spectra were compared with the mass spectral data of reference standards or those from NIST 05, Wiley 6, NBS 75 k, and in-house Flavorlib libraries.

2,2-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity assay

The DPPH radicals scavenging capacity of the essential oil was determined using a reported method [35]. Briefly, the samples of essential oil (1-10 mg/mL in ethanol) were mixed with 0.1 mM of DPPH reagent solution (Sigma Aldrich, St. Luis, MO, USA). Absorbance at 515 nm was measured after 30 min in dark. Eugenol (Sigma Aldrich, St. Luis, MO, USA) was used as standard. Radical scavenging capacity was expressed as inhibition percentage.

Ferric-reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was measured as previously described [36]. Briefly, the FRAP reagent was obtained by mixing 10 mM TPTZ reagent (2,4,6-tripyridyl-s-triazine, Sigma Aldrich) with ferric chloride (HCl, 40 mM and $FeCl_3{\bullet}6H_2O$, 20 mM) in acetate buffer (300 mM, pH = 3.6) at ratio of 1:1:10, respectively. The essential oil concentrations (30 µL) were mixed with 900 µL of ferric complex.

Statistical analysis

Agglomerative hierarchical cluster analysis of the 15 major components of *T. filifolia* essential oil compositions was carried out using SPSS Statistics, version 21 (IBM Corp., Chicago, IL, USA). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method. Fifteen major compounds were selected as variables: a-pinene, 1,8-cineole, (*E*)-b-ocimene, menthone, isomenthone, estragole, anisaldehyde, cuminaldehyde, (*Z*)-anethole, b-maaliene, methyl eugenol, (*E*)-b-ionone, b-bisabolene and spathulenol.

RESULTS AND DISCUSSION

The extraction yield was 0.67 % v/w, which was higher than 0.3 % from plants grow in Yucatan, Mexico [20], 0.47 % in Cusco, Peru [23] and 0.26 % in Venezuela [32], but lower than 0.63-1.31 % from aerial parts from plant grown at different heights in Argentina [21] and 12 % from leaves collected in Bologna, Italy [25].

Figure 1 shows the gas chromatogram on DB-5MS column of the essential oil. Quantitative data expressed as average percentage values and standard deviations of the constituents in the essential oil are listed in Table 1, according to the analyses in two columns with different polarities. Regarding the chemical composition, the essential oil was characterized by a high content of aromatic compounds (98.8 %), particularly phenylpropanoids. Among them (*E*)-anethole (85.0 %) and estragole (12.9 %) were the major constituents. All the other compounds were below 0.5 %. This high content

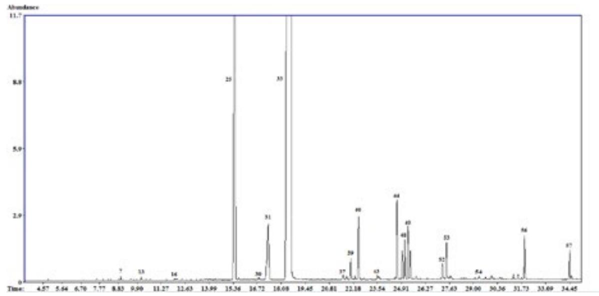

Figure 1. *Chromatographic profile on DB-5MS column of the essential oil from Tagetes filifolia aerial parts.*

of phenylpropanoids in comparison with those of the terpenic compounds is evidently a consequence that the biosynthetic pathway involving L-phenylalanine and cinnamic acid as precursors was favored in place of the terpenic biosynthetic route[37]. In contradistinction to terpenes, for which wide information is available about their biosynthesis, the entire biosynthetic pathways leading to the formation of phenylpropanoids and their derivatives still remains unclear [38]. The evolutionary relevance of plant volatile compounds awaits further investigations.

The data presented herein agree, at least in part, with some reports [13, 14, 18, 21, 22, 26-33] and differ from others [20, 23]. Some of these differences including that estragole was found the main compound [20, 24, 25] and (*Z*)-anethole was reported the major compound in place of (*E*)-anethole [23]. Nevertheless, it is well known that several factors such as crop growing location, plant development stage, harvest period, cultivation practices, parts of plant used for oil isolation, among others [39] can influence the oil content and the quantitative ratios of the oil components within the same species.

Although the chemical composition of essential oils can unfortunately be different depending on factors mentioned before, as a tentative general information is useful to know the different composition reported

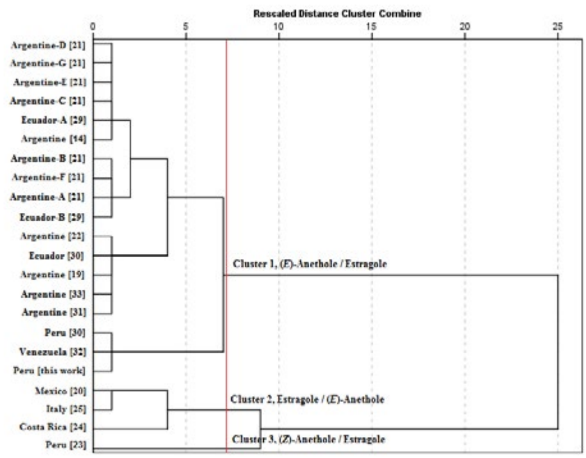

Figure 2. *Dendrogram obtained from hierarchical cluster analysis of Tagetes filifolia essential oil compositions.*

Table 1. *Composition of the essential oil from Tagetes filifolia aerial parts*

Peak	Compound	$RI_A{}^{a}$	$RI_A{}^{S\,b}$	$RI_P{}^{e}$	$RI_P{}^{S}$	%
1	(*E*)-2-Hexenal	853	856	1218	1219	tr[c]
2	Anisole	915	918	1308	1306	tr
3	a-Thujene	934	930	1011	1019	tr
4	Benzaldehyde	961	960	1508	1509	tr
5	Sabinene	972	975	1117	1115	tr
6	6-Methyl-5-hepten-2-one	983	986	1320	1323	tr
7	Octanal	1098	999	1296	1295	tr
8	(*E*)-3-Hexenyl acetate	1000	1002	1019	1018	tr
9	a-Phellandrene	1003	1003	1180	1177	tr
10	*p*-Cymene	1023	1025	1261	1265	tr
11	Limonene	1028	1029	1200	1195	tr
12	(*Z*)-b-Ocimene	1039	1037	1230	1232	tr
13	Phenylacetaldehyde	1040	1042	1650	1648	tr
14	Dihydrotagetone*	1051	1053	1266	-	tr
15	g-Terpinene	1057	1060	1258	1254	tr
16	Linalool	1096	1097	1555	1557	tr
17	Nonanal	1100	1101	1403	1400	tr
18	(*E*)-Tagetone*	1140	1144	1500	1501	tr
19	Camphor	1145	1146	1494	1497	tr
20	*trans*-Menthone	1150	1153	1473	1473	tr
21	(*E*)-2-Nonenal	1157	1162	1535	1538	tr
22	Isomenthone	1162	1163	1468	1465	tr
23	Terpinen-4-ol	1172	1177	1601	1606	tr
24	Naphthalene	1182	1181	1765	1763	tr
25	Estragole	1195	1196	1660	1655	12.9 ± 1.1
26	Decanal	1203	1202	1512	1515	tr
27	b-Cyclocitral*	1218	1220	1625	1624	tr
28	b-Citronellol	1223	1226	1755	1750	tr
29	Thymol methyl ether	1233	1235	1579	1577	tr
30	Pulegone	1238	1237	1648	1644	tr
31	*p*-Anisaldehyde	1250	1250	2025	2027	0.1 ± 0.0
32	(*Z*)-Anethole	1255	1253	1735	1732	tr
33	(*E*)-Anethole	1287	1285	1839	1837	85.0 ± 8.3
34	(*E,Z*)-2,4-Decadienal	1290	1293	1748	1752	tr
35	(*Z*)-3-Hexenyl tiglate*	1322	1325	1644	1647	tr
36	Piperitenone	1340	1343	1915	1918	tr
37	Modheph-2-ene*	1382	1384	1500	1499	tr
38	a-Isocomene	1290	1388	1540	1542	tr
39	Methyl eugenol	1403	1406	2034	2031	0.2 ± 0.0
40	2,5-Dimethoxy-*p*-cymene*	1423	1427	1916	1918	0.2 ± 0.0
41	(*E*)-Isoeugenol	1450	1451	2370	2372	tr
42	a-Humulene	1454	1455	1676	1670	tr
43	Geranyl acetone	1457	1455	1860	1865	tr
44	Germacrene D	1485	1485	1699	1690	tr
45	(*E*)-b-Ionone	1488	1489	1925	1923	0.4 ± 0.0

Table 1. (***continued***)

Peak	Compound	$RI_A{}^{a}$	$RI_A{}^{S\,b}$	RI_P	$RI_P{}^{S}$	%
46	Anisyl acetone*	1490	1493	2230	2234	0.2 ± 0.0
47	Bicyclogermacrene	1497	1500	1700	1698	± 0.0
48	(*E,E*)-a-Farnesene	1501	1506	1759	1755	tr
49	b-Bisabolene	1508	1506	1718	1715	tr
50	d-Cadinene	1520	1523	1764	1762	tr
51	(*Z*)-Nerolidol	1536	1533	2000	2005	tr
52	1-(4-Methoxyphenyl) propane-1,2-diol*	1566	1568	-	-	0.2 ± 0.0
53	Spathulenol	1580	1578	2134	2131	tr
54	*epi*-a-Cadinol	1646	1640	2174	2177	tr
55	a-Muurolol	1649	1646	2189	2182	tr
56	Nootkatone	1809	1807	2567	2563	0.2 ± 0.0
57	(*Z*)-Phytol	1950	1947	2576	2570	tr
	Aliphatic compounds					tr
	Aromatic compounds					98.8
	Monoterpene hydrocarbons					tr
	Oxygenated monoterpenes					0.4
	Sesquiterpene hydrocarbons					0.1
	Oxygenated sesquiterpenes					0.2
	Others					tr
	Total identified					99.5

[a]RI_A and RI_P, experimental linear retention indices on DB-5MS and DB-Wax column, respectively.
[b]$RI_A{}^S$ and $RI_P{}^S$, linear retention indices from standard or literature on DB-5MS and DB-Wax column, respectively.
*[c]trace (<0.1%). *tentatively identified compound by comparison with literature data.*

in all published studies. Agglomerative hierarchical cluster analysis was applied to examine the relationship between the studied sample and other *T. filifolia* specimens from 21 essential oil compositions reported in the literature[1 3, 14, 18-21, 23-25, 29-33]. Three different groups can be observed in the dendrogram shown in Figure 2. The first group was the most numerous and included samples with high level of (*E*)-anethole and lower levels of estragole. Cluster 2 included three samples from Mexico [20], Costa Rica [24] and Italy [25], which were dominated by high amount of estragole and lower amounts of (*E*)-anethole. The outlier cluster 3 was a (*Z*)-anethole/estragole group including a sample from Peru [23].

The antioxidant capacity of essential oils may vary depending on their chemical composition and the tests evaluated. Two in vitro methods were used in the present study, which previously have been used to predict the antioxidant properties of several substances (DPPH free radical scavenging test and ferric reducing antioxidant power (FRAP) assay). As shown in Table 2, the essential oil from aerial parts of *T. filifolia* exhibited radical scavenging activity at the highest concentrations testing (5 and 10 mg/mL). The percentages of DPPH inhibition were found in the order of 14 and 22 %, respectively. An essential oil from aerial parts of *T. filifolia* was reported to exhibit DPPH scavenger capacity with a mean effec-

Table 2. *Antioxidant activity of the essential oil from Tagetes filifolia aerial parts using DPPH and FRAP assays*

Sample	Concentration (mg/mL)	DPPH inhibition (%)	FRAP assay (μM of ascorbic acid equivalents)
Essential oil	10	22.7 ± 1.4	190.7 ± 7.1
	5	14.2 ± 0.9	NE
	1	NE	NE
Eugenol	0.1	60.1 ± 1.2	413.4 ± 8.2

Values are expressed as mean ± SD of three independent experiments. NE: not effect. DPPH: 2, 2-diphenyl-1-picryl-hydrazyl free radical scavenging assay, FRAP: ferric reducing antioxidant power. Eugenol was used as positive control.

tive concentration (EC_{50}) value of 20.2 mg/mL [18]. It has also been reported the different antioxidant capacities by DPPH assay of *Croton zehntneri* essential oils from the bark or leaf with EC_{50} values of 27 and 36 mg/mL, respectively. A gas chromatography analysis evidenced that the essential oils contain majority amounts of estragole and (*E*)-anethole [40]. Another study shows information on the free radical scavenging activities of fennel seed essential oil from Egypt and China, which demonstrated significant variations in the antioxidant properties according with their (*E*)-anethole composition [41]. Thus, the appreciable DPPH scavenging activity of *T. filifolia* essential oil could be due to its relative high amount of estragole and (*E*)-anethole.

In addition, the data revealed a reductive capacity for the *T. filifolia* essential oil at the highest concentration analyzed with the FRAP assay (Table 2), which means that the described essential oil has a moderate action on reducing/oxidant power of the ferric chloride. As was expected, eugenol showed stronger scavenger capacity of DPPH radical and ferric reduced power.

The essential oil antioxidant capacity could be explained to majority presence of (*E*)-anethole and estragole in their composition. An essential oil rich in (*E*)-anethole obtained by hydrodistillation of fresh *Clausena harmandiana* leaves containing 91.44 % (*E*)-anethole and 2.98 % estragole showed activity to scavenge DPPH (EC_{50} value of 18.48 mg/mL) and had the ability to reduce Fe^{3+}-TPTZ complex by FRAP assay [42]. To obtain a comprehensive measure of the overall antioxidant efficiency of the *T. filifolia* essential oil, it would be necessary to perform other studies to assess such properties on other experimental models.

CONCLUSIONS

Essential oil composition from aerial parts (leaves and flowers) of *Tagetes filifolia* grown in the Andean region of Peru show the presence of 57 volatile compounds, of which the most prominent were (*E*)-anethol (85.0%) and estragole (12.9%). The essential oil had a moderate antioxidant activity by using the DPPH and the ferric reducing antioxidant power assays**.** The volatile compounds profiles of *T. filifolia* specimens from 21 essential oil compositions can be divided into two groups: anethole/estragole and estragole/anethole, depending of which one is the major component.

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REFERENCES

1. Salehi, B.; Valussi, M.; Morais-Braga, M. F.B.; Pereira-Carneiro, J. N.; Alves-Borges Leal, A. L.; Melo-Coutinho, H. D.; Vitalini, S.; Kregiel, D.; Antolak, H.; Sharifi-Rad, M.; Cirone-Silva, N. C.; Yousaf, Z.; Martorell, M.; Iriti, M.; Carradori, S.; Sharifi-Rad J. *Tagetes* spp. essential oils and other extracts: Chemical characterization and biological activity. *Molecules* **2018**, 23, e2847.
2. Devika, R.; Koilpillai, J. Anti-inflammatory effect of bioactive compounds of *Tagetes erecta* (Linn.) flower extract. *J. Pure App. Microbiol.* 2015, 9, 2547-2551.
3. Kashif, M.; Bano, S.; Naqvi, S.; Faizi, S.; Lubna, S.A.; Mesaik, M.; Azeemi, K.S.; Farooq, A.D. Cytotoxic and antioxidant properties of phenolic compounds from *Tagetes patula* flower. *Pharm. Biol.* **2015**, 53, 672-681.
4. Padalia, H.; Chanda, S. Antimicrobial efficacy of different solvent extracts of *Tagetes erecta L.* flower, alone and in combination with antibiotics. *Appl. Microbiol.* **2015**, 1(1), e 1000106.
5. Politi, F. A.; Nascimento, J. D.; da Silva, A. A.; Moro, I. J.; García, M.L.; Guido, R. V.; Pietro, R.C.; Godinho, A. F.; Furlan, M. Insecticidal activity of an essential oil of *Tagetes patula* L. (Asteraceae) on common bed bug *Cimex lectularius* L. and molecular docking of major compounds at the catalytic site of clache1. *Parasitol. Res.* **2017**, 116, 415-424.
6. Sánchez-Humala, R.; Ruiz-Briceño, A.M.; Ruiz-Burneo, C. G.; Ruiz-Castro, G. M.; Sairitupac-Paredes, D. R.; Aguirre, L. G.; Salazar-Granara, A.; Loja-Herrera, B. Antioxidant activity and phytochemical screening of capitula of *Tagetes filifolia* Lag. or "pacha anís". *Horizonte Médico* **2017**, 17(1), 18-24.
7. Hammond, G. N.; Fernández, I. D.; Villegas, L. F.; Vaisberg, A. J. A survey of traditional medicinal plants from the Callejón de Huaylas, Department of Ancash, Peru. *J. Ethnopharmacol.* **1998**, 61, 17-30.
8. Moreno-Salazar, S. F.; Robles-Zepeda, E. E.; Johnson, D. E. Plant folk medicines for gastrointestinal disorders among the main tribes of Sonora, Mexico. *Fitoterapia* **2008**, 79, 132-141.
9. Bussmann, R.; Glenn, A. Plants used for the treatment of gastro-intestinal ailments in northern Peruvian ethnomedicine. *Arnaldoa* **2010**, 17, 255-270.

10. Svetaz, L.; Zuljan, F.; Derita, M.; Petenatti, E.; Tamayo, G.; Cáceres, A.; Cechinel-Filho, V.; Giménez, A.; Pinzón, R.; Zacchino, S. A.; Mahabir, G. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. *J. Ethnopharmacol.* **2010**, 127, 137-158.
11. Gheno-Heredia, Y.; Nava-Bernal, G.; Martínez-Campos, A.; Sánchez-Vera, E. Las plantas medicinales de la organización de parteras y médicos indígenas tradicionales de Ixhuatlancillo, Veracruz, México y su significancia cultural. *Polibotánica* **2011**, 31, 199-251.
12. Soukup, J. Vocabulario de los Nombres Vulgares de la Flora Peruana y Catálogo de los Géneros. Editorial Salesiana, Lima, **1987**.
13. Camiletti, B. X.; Asensio, C. M.; Gadbane, L. C.; Giménez-Pecci, M. P.; Conles, M. Y.; Lucini, E. I. Essential oils and their combinations with iprodione fungicide as potential antifungal agents against withe rot (*Sclerotium cepivorum* Berk) in garlic (*Allium sativum* L.) crops. *Ind. Crops Prod.* **2016**, 85, 117-124.
14. Zygadlo, J. A.; Guzmán, C. A.; Grosso, N. R. Antifungical properties of the leaf oils of *Tagetes minuta* L. and *T. filifolia* Lag. *J. Essent. Oil Res.* **1994**, 6(6), 617-621.
15. Cubillo, D.; Sanabria, G.; Hilje, L. Evaluación de la repelencia y mortalidad causada por insecticidas comerciales y extractos vegetales sobre *Bemicia tabaci. Manejo Integrado de Plagas* **1999**, 53, 65-72.
16. Serrato-Cruz, M. Á. Aspectos del cultivo de dos especies de *Tagetes* productoras de aceites esenciales. *Revista Naturaleza y Desarrollo* **2003**, 1(1), 15-22.
17. Camarillo, G.; Ortega, L. D.; Serrato-Cruz, M. Á.; Rodríguez, C. Actividad biológica de *Tagetes filifolia* (Asteraceae) en *Trialeurodes vaporariorum* (Hemiptera: aleyrodidae). *Rev. Colomb. Entomol.* **2009**, 35, 177-184.
18. Ruiz, C.; Cachay, M.; Domínguez, M.; Velásquez, C.; Espinoza, G.; Ventosilla, P.; Rojas, R. Chemical composition, antioxidant and mosquito larvicidal activities of essential oils from *Tagetes filifolia, Tagetes minuta* and *Tagetes elliptica* from Perú. *Planta Med.* **2011**, 77, PE30.
19. Gleiser, R. M.; Bonino, M. A.; Zygadlo, J. A. Repellence of essential oils of aromatic plants growing in Argentina against *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.* **2011**, 108, 69-78.
20. Bohrmann, H.; Youngken Jr, H. W. Esdragole, the main compound in the volatile oil of *Tagetes filifolia* (Compositae). *Phytochem.* **1968**, 7, 1415-1416.
21. Maestri, D. M., Zygadlo, J. A., Grosso, N. R., Aburra, R. E. and Guzman, C. A. Essential oil composition in *Tagetes filifolia* Lag. (Compositae) populations. *Anales de Biología* **1991**, 17(6), 133-136.
22. Zygadlo, J. A.; Lamarque, A. L.; Maestri, D. M.; Guzman, C. A.; Grosso, N. R. Composition of the inflorescence oils of some *Tagetes* species from Argentina. *J. Essent. Oil Res.* **1993**, 5(6), 679-681.
23. De Feo, V.; Della Porta, G.; Urrunaga-Soria, E.; Urrunaga-Soria, R.; Senatore, F. Composition of the essential oil of *Tagetes filifolia* Lag. *Flav. Fragr. J.* **1998**, 13, 145-147.
24. Vila, R.; Iglesias, J.; Canigueral, S.; Ciccio, J. F. Essential oil of *Tagetes filifolia* from Costa Rica. *Ingeniería y Ciencia Química* **2000**, 19(1), 13-14.
25. Marotti, M.; Piccaglia, R.; Biavati, B.; Marotti, I. Characterization and yield evaluation of essential oils from different *Tagetes* species. *J. Essent. Oil Res.* **2004**, 16(5), 440-444.
26. Serrato-Cruz, M. Á.; Díaz-Cedillo, F.; Barajas-Pérez, J. S. Seasonal influence on phenology and essential oil content of *Tagetes filifolia* Lag. *Annalen der Meteorologie* **2005**, 41(1), 82-85.
27. Serrato-Cruz, M. Á.; Díaz-Cedillo, F.; Barajas-Pérez, J. S. Composition of essential oil in germplasm of *Tagetes filifolia* Lag. from Central-South region of México. *Agrociencia* **2008**, 42, 277-285.
28. Vázquez, M.; Aimar, M. L.; Demmel, G. I.; Cabalen, M. E.; Criado, S. G.; Cantero, J. J.; Velasco, M. I.; Rossi, L. I. Determination of volatile organic compounds of *Tagetes filifolia* Lag. (Asteraceae) from Córdoba (Argentina) using HS-SPME analysis. *Bol. Latinoam. Caribe Plantas Med. Aromat.* **2013**, 12(2), 143-149.
29. Zapata-Maldonado, C. I.; Serrato-Cruz, M. Á.; Ibarra, E.; Naranjo-Puente, B. Chemical compounds of essential oil of *Tagetes* species of Ecuador. ECORFAN *Journal-Republic of Nicaragua* **2015**, 1(1), 19-26.
30. Ruiz, S.; Malagón, O.; Zaragoza, T.; Valarezo, E. Composition of the essential oils of *Artemisia sodiroi* Hieron, *Siparuna eggersi* Hieron, *Tagetes filifolia* Lag. and *Clinopodium nubigenum* (Kunth) Kuntze from Loja Ecuador. *J. Essent. Oil Bear. Plants* **2010**, 13(6), 676-691.
31. López, S. B.; López, M. L.; Aragón, L. M.; Tereschuk, M. L.; Slanis, A. C.; Feresin, G. E.; Zygadlo, J. A.; Tapia, A. A. Composition and anti-insect activity of essential oils from *Tagetes* L. species (Asteraceae Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug. *J. Agric. Food Chem.* **2011**, 59, 5286-5292.
32. Armas, K.; Rojas, J.; Rojas, L.; Morales, A. Comparative study of the chemical composition of five *Tagetes* species collected in Venezuela. *Nat. Prod. Comm.* **2012**, 7(9), 1225-1226.
33. Gadban, L. C.; Camiletti, B. X.; Bigatton, E. D.; Distéfano, S. G.; Lucini, E. I. Combination of *Tagetes filifolia* Lag. essential oil with chemical fungicides to control *Colletotrichum truncantum* and their effects on the biocontrol agent *Trichoderma harzianum. J. Plant Prot. Res.* **2020**, 60(1), 41-50.
34. Costa, R.; Zellner, B. D. A.; Crupi, M. L.; De Fina, M. R.; Valentino, M. R.; Dugo, P.; Dugo, G.; Mondello, L. GC-MS, GC-O and enantio-GC investigation of the essential oil of *Tarchonanthus camphoratus* L. *Flav. Fragr. J.* **2008**, 23, 40-48.
35. Tabart, J.; Kevers, C.; Pincemail, J.; Defraigne, J.-O.; Dommes, J. Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chem.* **2009**, 113, 1226-1233.

36. Benzie, I. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, 239(1), 70-76.
37. Eslahi, H.; Fahimi, N.; Sardarian, A. R. Chemical composition of essential oils. In: Essential Oils in Food Processing: Chemistry, Safety and Applications. Hashemi, S. M. B., Khaneghah, A. M. and de Souza Sant'Ana, A. (eds). Wiley-Blackwell Chichester, pp. 119-172, **2017**.
38. Dudareva, N.; Negre, F.; Nagegowda, D. A.; Orlova, I. Plant volatiles: Recent advances and future perspectives. *Crit. Rev. Plant Sci.* **2006**, 25, 417-440.
39. Almeida-Barbosa, L. C.; Andrade-Filomeno, C.; Teixeira, R. R. Chemical variability and biological activities of *Eucalyptus* spp, essential oils. *Molecules* **2016**, 21(12), e1671.
40. Donati, M.; Mondin, A.; Chen, Z.; Mendes, F.; Braga, B.; Schirato, G.; Pastore, P.; Froldi, G. Radical scavenging and antimicrobial activities of *Croton zehntneri, Pterodon emarginatus* and *Schinopsis brasiliensis* essential oils and their major constituents: estragole, trans-anethole, β-caryophyllene and myrcene. *Nat. Prod. Res.* **2015**, 29(10), 939-946.
41. Ahmed, A. F.; Shi, M.; Liu, C.; Kang, W. Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. *Food Sci. Hum. Wellness* **2019**, 8(1), 67-72.
42. Peerakam, N.; Phoowiang, P.; Chansakaow, S.; Thongpoon, Ch.; Hadpech, S. Chemical profiling revealed a dominant compound trans-anethole and biological evaluation of an edible plant *Clausena harmandiana* containing essential oil. *Rec. Nat. Prod.* **2021**, 16(2), 118-122.