UNIVERSIDAD AUTÓNOMA DE GUERRERO

FACULTAD DE CIENCIAS QUÍMICO BIOLÓGICAS
FACULTAD DE CIENCIAS DE LA TIERRA

MAESTRÍA EN BIOCIENCIAS

COMPOSICIÓN QUÍMICA, ACTIVIDAD ANTIOXIDANTE Y ANTIGENOTOXICA DE ACEITES ESENCIALES DE *Porophyllum ruderale* Y *Porophyllum tagetoides*

T E S I S

PARA OBTENER EL GRADO DE

MAESTRA EN BIOCIENCIAS

PRESENTA

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APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 20 días del mes de febrero de dos mil dieciocho, se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado de la Maestría en Biociencias, para examinar la tesis titulada "**Composición química, actividad antioxidante y antigenotóxica de aceites esenciales de *Porophyllum ruderale* y *Porophyllum tagetoides***", presentada por la alumna **Ana Karen Villa Merlán**, para obtener el Grado de Maestría en Biociencias. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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INDICE

Phytochemical characterization, anti-oxidant and anti-genotoxic activity of the essential oils from *Porophyllum ruderale* subp *macrocephalum* and *Porophyllum tagetoides*

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Summary

Porophyllum tagetoides y *Porophyllum ruderale* subp *macrocephalum* son plantas utilizadas en la medicina tradicional mexicana. El objetivo de este estudio fue caracterizar la composición química de los aceites esenciales de *P. tagetoides* y *P. ruderale* subp *macrocephalum*, su capacidad anti-oxidante y anti-genotóxico. El análisis cuantitativo y cualitativo de los aceites esenciales (AE) se realizó utilizando CG-MS. La capacidad anti-oxidante fue cuantificada por los métodos de $DPPH^{\cdot}$ y $ABTS^{\cdot}$ y la capacidad anti-genotóxica mediante el ensayo de micronúcleos con bloqueo de la citocinesis. Los AE extraídos de las hojas de *P. ruderale* subp *macrocephalum y P. tagetoides* (frescos y secos), mostraron que poseen capacidad anti- oxidante ya que poseen un porcentaje de inhibición del 71.31% del radical $ABTS^{\cdot}$. Además, el pre-tratamiento con los AE disminuyó la frecuencia de micronúcleos, lo que sugiere un efecto protector al ADN y esto puede estar relacionado con el D- limoneno, que fue uno de los compuestos mayoritarios en los AE. Los AE tienen capacidad anti-oxidante y anti-genotóxica.

Palabras clave: *Porophyllum tagetoides*, *Porophyllum ruderale* subp *macrocephalum*, D-limoneno, perfil fitoquímico, anti-oxidante, anti-genotóxico.

Abstract

Porophyllum tagetoides y *Porophyllum ruderale* subp *macrocephalum* are used plants in traditional Mexican medicine. The aims of this study were to characterize chemical composition of the essential oils from *P. tagetoides* y *P. ruderale* subp *macrocephalum,* their anti-oxidant and anti-genotoxic activities. The quantitative and qualitative analyses of the essential oils (EO) were performed by means of GC-MS. The anti-oxidant capacity was quantified by the methods DPPH· and ABTS· and the anti-genotoxic capacity through the micronucleus assay with blockade of cytoquinesis. The EOs extracted from leaves of *P. ruderale* subp *macrocephalum y P. tagetoides* (fresh and dry), showed that they possess anti-oxidant capacity since they have of 71.31% a inhibition percentage of free radicals, moreover , pre-treatment with OE decreased the frequency of micronuclei, suggesting a protective effect for DNA and this may be related to D-limonene, which was one of the major compounds in the OE. The OEs have anti-oxidant and anti-genotoxic properties.

Keywords*: Porophyllum tagetoides*, *Porophyllum ruderale* subp *macrocephalum*, D-Limonene, phytochemical profile, anti- oxidant, anti-genotoxic.

Introduction

The Asteraceae family consists of 1600 genus [1] and it is characterized by its therapeutic and pharmacological properties [2]. The most representative genus of this family are *Mikania, Vernonia* and *Porophyllum* [3]. Due to their therapeutic use, the genus *Porophyllum* has approximately 28 important species [4]; between these species *Porophyllum tagetoides* (pipisa) and *Porophyllum ruderale* subp *macrocephalum* (pápalo) are the main [5]. These two species are commonly used in folkloric Mexican cuisine. Their leaf are edible, and they are used for food seasoning [6]. These species are characterized by their fragrances. In addition, they are use in traditional medicine as anti-inflammatory [7], healing [8] and bruises treatment [9]; however, the pharmacological studies are limited. In the sense, anti-parasitic and antioxidant activities have been reported for crude extracts [10]. Regarding the phytochemical constitution of their extracts, different monoterpenes and sesquiterpenes have been identified [5].

The medicinal potential of plants has recently regained importance and also it has aroused scientific interest. In recent years, a variety of extracts, fractions, pure compounds and essential oils (EO) have been tested showing a broad range of biological activities such as anti-proliferative [11], anti-bacterial [12] and anti-oxidant [13]. In particular, it interesting the use of EOs, which are characterized by their smell and yellowish color [14]. They are mixtures that can contain from 20 to 260 compounds in diverse concentrations [15, 16], which may confer them their diverse biological effects. Into these beneficial effects, the anti-oxidant activity results quite important because of its association to prevention of diseases caused by DNA damage and /or oxidative stress. These diseases include various types of cancer [17], diabetes [18], cardiovascular disorders and mutagenesis [19]. Although most organisms have some anti-oxidant

defense and reparation systems, sometimes they are not enough to avoid the disease state. Because of this, the interest for natural products such as plant extracts to replace synthetic anti-oxidants has recently increased [15]. Therefore, the aims of this study were to characterize the chemical composition of the essential oils from *Porophyllum tagetoides* and *Porophyllum ruderale* subp *macrocephalum*, as well as their anti-oxidant and anti-genotoxic activity.

Materials and methods

Plant material

Leaf from *Porophyllum ruderale* subp *macrocephalum* and *Porophyllum tagetoides* were collected at the municipality of Chilapa de Álvarez, Guerrero (between parallels 17° 17' 40" and 17° 40' 55" North latitude, and 98° 53' 44" and 99° 17' 11" West longitude) in August 2016. Half of the collection was dehydrated in a stove with forced air recirculation at 40 °C. Also, fresh material was stored at -80 °C until the extraction of EOs. The specimens were deposited in the Herbarium of the Ethnobotanical Garden of the National Institute of Anthropology and History (INAH), Cuernavaca, Morelos, Mexico. The specimens were identified by the Biologists Margarita Aviles and Macrina Fuentes. The specimens correspond to *Porophyllum ruderale* (Jacq) Cass subsp *macrocephalum* (INAHM 2072) and *Porophyllum tagetoides* (Kunth) Dc (INAHM 2073).

Essential oils extraction

The extraction of the EO was done by means of hydrodistillation, using a Clevenger like system in the same way as Sartoratto et al. [20]. Briefly, the plant material was placed in a 2 L round bottom flask together with 500 mL of distilled water. Then the plant material was boiled for 3 h. After, the hydrolate was recovered and it was liquid-liquid extracted with dichloromethane (20 mL) for three times. The aromatic extract was evaporated on a rotary evaporator (Büchi B-480®) at 40 °C. When as much as possible solvent was evaporated, the remaining oil was transferred to a 1.5 mL amber glass vial. The rest of the solvent was evaporated at room temperature in a fume hood. The EOs in were stored at -80 °C until their chemical analysis. The yield of EOs was calculated with the following formula: yield = (g of oil obtained X 100) / g of starting plant material. The yield results were expressed as a percentage value.

Gas chromatography coupled to mass spectrometry (GC-MS)

The EOs were analyzed by GC-MS with a HP 6890 Series Hewlett Packard chromatograph coupled to an HP 5973 mass detector. The GC-MS system used an HP 5-M/30 mx 0.250 μm column and 0.25 μm thickness. Helium was used as a carrier gas with 99.9% of purity at a flow of 1 mL/min. The oven temperature started at 40 °C and increased 5°C /min until reaching a temperature of 220 °C. The temperature of the injector in "splitless" mode was 220 °C, and 280 °C as the interface temperature. For the mass detector in SCAN mode, the temperature of the ion source was 230 °C, and that of the quadrupole of 150 °C. The ionization energy was 70 eV in electronic impact mode. The injection volume was 1 μL of an essential oil dilution with dichloromethane (1:1000 v/v). The identification of the compounds was made by comparing the mass spectra of the samples with those of the NIST V. 2002 Library, or by comparing the retention time of the compounds present in the samples with those of standard references.

Anti-oxidant activity

1,1-Difenil-2-picrilhidrazilo ($DPPH^{\cdot}$)

It was carried out according to the methodology described by Salaiman et al. [21]. Briefly, 50 μL of the EOs (25-100 μg/mL) were added to 96-well plates and then 150 μL of $DPPH^{\cdot}$ were subsequently added in the well. The controls were dimethylsulfoxide (DMSO, 50μL) and $DPPH^{\cdot}$ solution (150μL). The oils-$DPPH^{\cdot}$ and the control solutions were incubated for 30 min at 37 °C in darkness. Subsequently, the absorbance of each well was measured at 545 nm in a microplate reader (STAT Fax 2100). The anti-oxidant potential was expressed as percentage of inhibition, and inhibitory concentration 50 (IC_{50}) values.

2,2'-Azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid (ABTS$^{\cdot}$)

This method was performed according to the methodology of Thaipong et al. [22]. Briefly, stock solutions of ABTS$^{\cdot}$ (7.4 mM) and potassium persulfate (2.6 mM) were mixed 1:1 (v/v). They react for 12 h at room temperature in dark conditions (Solution A). Subsequently, an analysis solution was made by diluting 1 mL of solution A, in 60 mL of methanol (solution B/ABTS$^{\cdot}$). The EOs solutions (50 µL) were placed in 96-well plates and 150 µL of the B solution were subsequently added in the well. The plates were incubated for 30 min at 37 °C in the dark. Subsequently, the absorbance measurement was done at 734 nm in a spectrophotometer (Thermo scientific). In the same way as for the DPPH$^{\cdot}$ method, the results were expressed in percentage of inhibition and total anti-oxidant capacity.

Anti-genotoxic assay

The Anti-genotoxic effect of the essential oils of *P. ruderale* and *P. tagetoides* was evaluated by the method proposed by Fenech [23] in peripheral blood mononuclear cells (PBMC). Primary cultures of PBMC from healthy male donors (n = 3) between 21 and 26 years of age, with no history of occupational exposure to pesticides, alcohol use, consumption of antioxidant supplements (vitamins), genetic diseases, smoking, or drug use were used. The participation of each subject was voluntary. Informed consent was obtained from the subjects according to the recommendations of the Helsinki declaration.

Peripheral blood mononuclear cells isolation

PBMCs were obtained using Ficoll-Paque-Premium (GE HEALTHCARE-**Sweden**). Briefly, venous blood was collected using vacutainer system with EDTA under asepsis conditions. Next, the Venous blood was centrifuged for 10 min at 424.8 g. After, the

leukocyte layer was extracted and transferred to 3 ml of PBS and centrifuged by 10 min at 271.9 g. The mixture was added carefully to Ficoll (2 mL), this mixture was centrifuged for 15 min at 329 g. Finally, the leukocyte layer was washed using 3 mL of PBS.

Micronuclei assay

PBMC were plated in RPMI-1640 (Gibco, Gran Island, N.Y.) medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% of nonessential amino acids and 0.007 mg/mL of phytohemaglutinin (PHA). The incubation was performed at 37 °C in a humidified atmosphere with 5% CO_2. PBMC were stabilized for a period of 24 h before each assay.

To measure the anti-genotoxic capacity of *P. ruderale* and *P. tagetoides* oils (fresh and dry) the PBMCs were exposed to 265 ug/mL of genotoxic agent Mitomicin C from *Streptomyces caespitosus* (Sigma-Aldrich), after, a hour the EOs were added (25 and 50 µg / mL).

After 48- or 24-h of incubation, the cytokinesis was stopped using 11.25µL of cytochalasin B [2 mg / ml] by 24 h under the previously described conditions. Cells were harvested, fixed with ice-cold fixative solution (methanol-acetic acid 3:1). Then the tubes were centrifuged for 10 min at 153 g. The supernatant was discarded and then the peeled was gently re-suspended with 1ml of fixative solution. This step was repeated 4 times. The peeled was recovered in 0.5 mL of fixative solution, this mixture were placed on a glass slide to make a smear. The dry slides were stained with Hemacolor dye (Merck, Germany). The micronucleus count was done under a compound microscope with a 100x objective. One thousand binucleated cells were counted to determine the frequency of micronucleus. To determine the concentration of essential oils used for the evaluation of their anti-genotoxic effects, increasing concentrations of 25, 50, 100, 200 and 400 µg / mL were tested. The concentration that no showed a cytotoxicity effect were chosen to

be tested against mit-C. The cellular viability was measured during 24 and 48h by the MTT and DFDA assays. In addition, the concentration used of (mit-C) no showed decrease in the cell viability, and their division index.

Statistical analysis

The results of essential oil yield and antioxidant activity determined by the method of DPPH and ABTS were expressed as mean ± standard deviation (n=3). The analysis of variance was carried out by means of an ANOVA test, and the grouping of means for the comparison of yields of essential oil and antioxidant activity was done by means of the Tukey test ($\alpha = 0.05$), for the micronuclei assay a descriptive analysis is carried out followed by an ANOVA test with Dunnett post hoc test.

Results

Plant material

Regarding the yield of essential oils extracted from fresh and dried leaves of *Porophyllum ruderale* subp *macrocephalum* (pápalo), the obtained yields were 0.00881 and 0.0807% respectively. In the case of *Porophyllum tagetoides* (pipisa), the yields for fresh and dry leaves were 0.04933 and 0.1213% respectively. In both cases, it was observed that the dry plant material provides a higher yield of essential oil. In the case of the pápalo, the yield of dry material was at least 10 times higher than the one from fresh material.

Analysis of the essential oils

By means of GC-MS analysis, differences in the chemical composition of the EOs extracted from fresh and dried leaves of *P. ruderale* and *P. tagetoides* were determined (Table 1). In general, within each species there are qualitative and quantitative differences between essential oils extracted from fresh and dry plant material.

In *P. ruderale* 11 compounds were detected in essential oils extracted from fresh leaf and 13 in those extracted from dried leaf. In the oils obtained from fresh leaves, 72.72% of the compounds corresponded to hydrocarbons, 18.18% to cyclic hydrocarbons, 9.09% to diterpenes and the remaining 0.01% was composed of trace metabolites. On the other hand, in the case of essential oil extracted from *P. ruderale* dried leaf, just the 46.15% corresponded to hydrocarbon compounds, 30.76% to cyclic hydrocarbons and monoterpenes, and finally 23.07% was composed by aldehydes, diterpenes and ketones.

Besides, in *P.tagetoides* oils extracted from fresh material, 11 metabolites were identified, from which 36.36% belong to the alcohols, 27.27% to aldehydes and 36.36% to mono- and sesquiterpenes. The oil extracted from dried leaf showed 13 compounds from which

the 46.15% corresponded to cyclic hydrocarbons and aldehydes, the 38.46% to aldehydes, alcohols, sesquiterpenes and ketones, and 15.38% to monoterpenes (Table 1). In both cases, the major peak corresponded to D-limonene.

Table 1. Chemical components in essential extracted from fresh and dry *P. ruderale* subsp *macrocephalum* and *Porophyllum tagetoides* leaves

Compound's name	RT (min)	Formula	Molecular mass	Score (%)	FPRm (%)	DPRm (%)	FPT (%)	DPT (%)
3-hexen-1-ol*	4.914	$C_6H_{12}O$	100.1	67	-	-	8.23	-
2-cyclohexen-1-one	6.677	C_6H_8O	96.1	86	-	-	-	1.34
menthol	7.410	$C_{10}H_{16}O_2$	168.1	63	1.56	-	-	-
menthomenthene	7.704	$C_{10}H_{16}$	136.1	85	-	2.32	-	-
2.3.5-trimethyl-4-methylene-2-cyclopenten-1-one	7.713	$C_9H_{12}O$	136.1	93	-	-	0.59	-
β-phellandrene	7.722	C10H16	136.1	70	-	-	-	1.89
trans-p-mentha-2.8-dienol	7.852	$C_8H_{16}O$	152.1	66	0.78	-	-	-
1-octen-3-ol	7.909	$C_8H_{16}O$	128.1	72	-	0.16	-	-
β-myrcene*	8.200	$C_{10}H_{16}$	136.1	68	-	0.63	-	-
pinocampheol	9.239	$C_{10}H_{18}O$	154.1	78	2.53	-	-	-
D-limonene*	9.393	$C_{10}H_{16}$	136.1	78	-	80.19	58.63	89.47
1.2-bis(1-methylethenyl)-trans-cyclobutane	9.682	$C_{10}H_{16}$	136.1	78	-	0.02	-	-
3-p-menthene	9.808	$C_{10}H_{16}$	136.1	82	-	0.14	-	-
2-methyl-6-methylene-octa-1.7-dien-3-ol	10.435	$C_8H_{16}O$	152.1	72	0.53	-	-	-
octyl-cyclopropane	10.981	$C_{11}H_{22}$	154.1	54	-	-	2.05	1.21
linalool*	11.233	$C_{10}H_{18}O$	154.1	85	-	0.53	-	-
tridecane	11.241	$C_{13}H_{28}$	184.2	73	1.38	-	-	-
nonanal	11.344	$C_9H_{18}O$	142.1	87	-	0.14	-	-
(*E*)-p-2.8-menthadien-1-ol	11.767	$C_{10}H_{16}O$	152.1	83	-	0.30	-	-
cis-(-)-1.2-epoxy-p-menth-8-ene	12.103	$C_{10}H_{16}O$	152.1	83	-	0.16	-	-
(*E*)-p-2-menthen-1-ol	12.140	$C_{10}H_{16}O$	152.1	81	0.51	0.19	-	-
(+)-(E)-limonene oxide	12.218	$C_{10}H_{16}O$	152.1	88	-	0.22	-	-
2.4.6-trimethyl-octane	12.709	$C_{11}H_{24}$	156.2	72	2.01	-	-	-
(E)-2-nonenal	12.757	$C_9H_{16}O$	140.1	87	-	0.14	-	-
5.5-dimethyl-hexanal.	12.872	$C_8H_{16}O$	128.1	60	0.58	-	-	-
α-farnesene*	13.186	$C_{15}H_{24}$	204.2	69	0.64	-	-	-
terpinen-4-ol	13.191	$C_{10}H_{18}O$	154.1	74	0.75	0.15	-	-
3.3.6-trimethyl-1.5-Heptadiene	13.536	$C_{10}H_{18}$	138.1	75	36.84	-	17.77	-
(E)-4-decenal	13.583	$C_{10}H_{18}O$	154.1	86	-	0.20	2.37	-
santolina triene	13.642	$C_{10}H_{16}$	136.1	76	-	0.05	-	-
(-)-trans-isopiperitenol	13.758	$C_{10}H_{16}O$	152.1	83	-	0.01	-	-

decanal	13.864	$C_{10}H_{20}O$	156.2	89	5.96	3.28	3.62	2.00
3-isopropyl-5-methyl-hex-4-en-2-one	14.054	$C_{10}H_{18}O$	154.1	74	-	0.25	-	-
carveol	14.184	$C_{10}H_{16}O$	152.1	78	0.99	0.26	-	-
carvone*	14.750	$C_{10}H_{14}O$	150.1	86	0.38	0.23	-	-
2.2-dimethyl-*(E)*-4-decene	15.224	$C_{12}H_{24}$	168.2	79	-	0.19	-	-
benzenepropanoic acid methyl ester	15.431	$C_{10}H_{12}O_2$	164.1	68	0.39	0.16	-	-
dihydroedulan	15.727	$C_{13}H_{22}O$	194.2	82	0.81	0.11	-	-
benzenepropanoic acid ethyl ester	16.948	$C_{11}H_{14}O_2$	178.1	84	0.62	0.37	-	-
(*Z.Z)*-3.6-nonadienal	17.825	$C_9H_{14}O$	138.1	82	1.27	0.73	-	-
caryophyllene*	18.376	$C_{15}H_{24}$	204.2	81	-	0.11	0.64	-
2.5.5-trimethyl-1.6-heptadiene	18.603	$C_{10}H_{18}$	138.1	80	2.05	0.42	0.92	-
2.6-dodecadien-1-al	18.858	$C_{12}H_{20}O$	180.2	82	-	7.76	-	4.09
3.3-dimethyl-hexane.	18.991	C_8H_{18}	114.1	78	0.77	-	-	-
trans-β-ionone	19.584	$C_{13}H_{20}O$	192.2	80	1.04	0.19	-	-
hexadecane	21.232	$C_{16}H_{34}$	226.3	73	0.78	-	-	-

*Compounds identified by standard retention time comparison. FPRm (Fresh *P. ruderale* subsp *macrocephalum*), DPRm (Dry *P. ruderale* subsp *macrocephalum*), FPT(Fresh *Porophyllum tagetoides*), DPT (Dry *Porophyllum tagetoides*).

Anti- oxidant of essential oils

The $DPPH^{\cdot}$ method measures the capacity of substances to stabilize this radical by donating electrons to this compound, for example, if the number of hydroxyl groups in a compound is high, then its anti-oxidant activity could be higher. The results of the antioxidant activity of *P. ruderale* and *P. tagetoides* are shown in Table 2.

Generally, fresh *P. ruderale* and dry *P. tagetoides* have the highest percentage of inhibition of the $DPPH^{\cdot}$ Radical. In other hand, the EOs obtained from leaf fresh and dry *P. ruderale* inhibit 71% of the $ABTS^{\cdot}$ radical. the EOs of from fresh *P. ruderale* presents IC_{50} lowers (297.07 DPPH and 5.735 ABTS).

Table 2.- Antioxidant effect of *P. ruderale subsp macrocephalum* and *P. tagetoides* in the technique of DPPH and ABTS

	% INH		Total antioxidant capacity mgEEAA/mL		IC_{50} mg /mL	
Aceite esencial	**DPPH**	**ABTS**	**DPPH**	**ABTS**	**DPPH**	**ABTS**
FPRm	14.351 ± 0.784^{a}	71.31 ± 0.669^{a}	85.269 ± 0.975	8.182 ± 1.36002	297.07^{a}	5.735^{a}
DPRm	8.101 ± 1.393^{b}	71.50 ± 1.0103^{a}	77.514 ± 1.72	9.358 ± 0.00377	478.45^{b}	6.538^{b}
FPT	5.26 ± 2.6905^{b}	53.96 ± 1.190^{b}	78.7715 ± 9.9364	7.2503 ± 0.0532	748.76^{b}	6.839^{b}
DPT	$16.238+ .819^{a}$	52.23 ± 1.574^{b}	90.7026 ± 4.4482	7.1428 ± 0.0651	351.55^{b}	6.835^{b}

FPRm (Fresh *P. ruderale* subsp *macrocephalum*), DPRm (Dry *P. ruderale*(Jacq) Cass subsp *macrocephalum*), FPT (Fresh *Porophyllum tagetoides*), DPT (Dry *Porophyllum tagetoides*). IC_{50} = Inhibitory concentration 50;% INH = Percentage of inhibition. The results are expressed as the mean ± standard deviation. The comparison was made with Anova one way with Tukey test. Different letters indicate significant difference ($p = <0.05$)

Anti-genotoxic activity

Concentrations of essential oils chosen to be used in the bioassay were 25 µg/mL and 50 µg/mL. These concentrations did not affect the cellular viability neither nuclear division index (1.2- 1.6) at 24 and 48 h (data not showed). The cells pre-treated with EOs and exposed to mit-C showed a clear decrease in the frequency of micronuclei in comparison with those cells without pre-treated with EOs. The EOs extracted from fresh leaf *P. ruderale* was able to reduce almost 96% ± 1.39 of the genotoxic damage in comparison with mit-C (Table 3) cells at 24 h. In the same way, the EOs extracted from fresh and dried material of *P. tagetoides* showed a decrease in the frequency of micronucleus at 24 and 48 h.

Table 3.- MN frequency at 24 and 48 h of exposure of the AEs to the MIT-C.

Treatment group	Dose (µg /mL)	24 h		48 h	
		MN/ 10^3 BN cell	**MN (%) ± S.D**	**MN/ 10^3 BN cell**	**MN (%) ± S.D**
CONTROL (-)	---	0.33 ± 0.51 [a]	2.78 ± 0	0.5 ± 0.54 [a]	2.60 ± 0
CONTROL (+)	2	36 ± 1 [b]	100 ± 2.78	38.33 ± 1.52 [b]	100 ± 3.98
FPRm	25	3 ± 1.78 [a]	8.33 ± 4.67	4.83 ± 1.47 [a]	12.60 ± 3.83
FPRm	50	1.5 ± 0.83 [a]	4.17 ± 2.32	3.66 ± 0.81[a]	9.56 ± 2.12
DPRm	25	3.33 ± 1.96 [a]	11.11 ± 3.40	3.66 ± 0.51[a]	9.56 ± 1.34
DPRm	50	4.66 ± 0.81 [a]	12.96 ± 2.27	5.66 ± 1.86 [a]	14.78 ± 4.85
FPT	25	3.33 ± 1.96 [a]	11.11 ± 3.40	5.16 ± 1. 16[a]	13.47 ± 3.04
FPT	50	2.66 ±1.63 [a]	7.41 ± 4.54	4.83 ± 1. 16[a]	12.60 ± 3.04
DPT	25	3.66 ± 1.96 [a]	10.19 ± 5.46	3.83 ± 0.75 [a]	10 ± 1.96
DPT	50	4.16 ± 1.16 [a]	11.57 ± 3.25	5.83 ± 1.94 [a]	15.21 ± 5.06

CONTROL (-) DMSO; CONTROL (+) mit -C ;FPRm (Fresh *P. ruderale* subsp *macrocephalum*), DPRm (Dry *P. ruderale* subsp *macrocephalum*), FPT(Fresh *Porophyllum tagetoides*), DPT (Dry *Porophyllum tagetoides*). The values represent the average ± S.D. Letter different significant statistically significant difference P = -0.05.

Discussion

Porophyllum ruderale and *Porophyllum tagetoides* are endemic plants of the so-called quelites. Due to their cosmopolite growth and its multiple empirical medicinal properties [24], they are often consumed in the state of Guerrero, Mexico. In the present study, the yields of essential oils extracted from fresh and dry leaf of *P. ruderale* and *P. tagetoides* were determined. In general, a leaf yield was gotten from dry materials in both species. This may be due to the water content in fresh tissues. It has been reported that the water can affect the extraction efficiency, since water is a mechanical barrier it can modify the entrance efficiency of the extracting solutions, therefore, the extracted components and its proportions are decreased [25].

As in the case of EOs yield differences between the two species, there were also differences in their chemical composition. In the EOs of *P. tagetoides* (fresh and dried) and in those extracted from *P. ruderale* dried, D-limonene was found as the major component, this compound has been previously reported as part of the aromatic composition of *P. ruderale*, although not as a major compound. About this, it has been

previously reported that the chemical diversity in essential oils varies according to the place of origin, weather, collection time, genetic structure, agricultural practices and the extraction method [26]. Therefore, it can be assumed that the conditions in which these specimens were growing mainly promote the production and accumulation of this compound in the essential oils analyzed. On the other hand, the differences observed in fresh and dry leaves can be explained due to fresh leaf are possess a higher content of cuticular waxes, which are matrixes that are characterized as complex mixtures of chemical components such as alkanes, esters, alcohols and long chain fatty acids. It has been observed that the drying of plant material can significantly increase the concentration of some compounds even up to 5.2%. Regarding the anti-oxidant activity, the results showed that the essential oil of *P. ruderale* has the highest capacity of inhibition of the tested radicals (14% $DPPH^{\cdot}$ and 71% $ABTS^{\cdot}$). Besides, the EOs of *P. tagetoides* also showed outstanding inhibitory activity values (16% $DPPH^{\cdot}$ and 52% $ABTS^{\cdot}$). It has been reported that the compounds present in EOs can participate as proton donors stabilizing oxygen and nitrogen free radicals [27], which supports the high effects of the tested oils.

In this context, it is well known that we are daily exposed to various genotoxic factors that affect our cellular integrity. The alteration of this integrity can cause various pathologies, so the interest in prevention above treatment has rapidly increased. The EOs of *P. ruderale* and *P. tagetoide*s, once in contact with the cells they generate a protective effect against the genotoxicity produced by mit-C. Concentrations of 25 and 50 µg/mL of EOs from fresh *P. ruderale* leaf produce a protective effect against the genotoxic agent. Furthermore, the anti-genotoxic effect significantly increased as the concentration increased. However it is worth to mention that at higher concentrations than 50 µg/mL of OEs a toxic effects was produced in the lymphocytic cells. Hence the geno-protective

activity of this essential oils is concentration-dependent (Supplementary material 1). Also, in the case of *P. ruderale* EOs there was a significant higher anti-genotoxic effect in the essential oils extracted from fresh leaf. However, this just occurs at 50 µg/mL. On the other hand, for *P. tagetoides* EOs extracted from fresh and dry leaf, there was no differences in their anti-genotoxic activity in both 25 and 50 µg/mL. In this regard [28], suggested that EOs or extracts, can activate the NRF2 pathway. Normally, NRF2 is linked to the KEAP1 complex and once the EOs enters the cell dissociates NRF2 from KEAP1, internalizing to the nucleus and activate the ARE promoter (antioxidant response element), producing the synthesis of enzymes such as Glutamate, Quinona oxidoreductose in others which in turn helps protect DNA damage, damage to proteins and mitochondria. Hence, it can be suggest that the EOs of *P. ruderale* and *P. tagetoides* can activate this pathway as one possible mechanism to protect the cell from the damage caused by mit-C. One more trend observed in this study was the significant decrement of the protective effect of EOs at 48 h. This can be due to the nature of the bioassays, cell cultures were carried out at 37 °C, which can cause EOs evaporation and parallel to this the gradual loss of their protective effects.

EOs have been characterized by their multiple uses as anti-parasitic, anti-inflammatory and anti-oxidant agents. This multi-usage feature is attributed to their most common compounds such as hydrocarbons, alcohols, aldehydes, ketones, phenols, oxides and esters [29]. The EOs of *P. ruderale* and *P. tagetoides*, present as major D-limonene in almost all their essential oils, which has been reported as a powerful anti-oxidant. Hence, D-limonene it is a good candidate to be tested in a pure form as an anti-genotoxic compound.

Conclusions

According to the results it can be concluded that EOs from *Porophyllum ruderale* subsp *macrocephalum* and *Porophyllum tagetoides* contain a great variety of chemical compounds and they are good anti-oxidant agents. Moreover, the essential oils from both species, regardless their treatment process, are potent anti-genotoxic substances. So, these quelites can come to be denominated like foods, Generally Recognized As Safe (GRAS), this by the properties that are attributed to him and the protective activities that they possess.

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Anexo 1

Identificación de *Porophyllum ruderale* subp *macrocephalum*

Ficha descriptiva	
NOMBRE CIENTIFICO	*P. ruderale*(Jacq) Cass subsp *macrocephalum*
FAMILIA	Asterácea
LOCALIDAD	Chilapa de Álvarez
LATITUD	17.6594
LONGITUD	-99.8672
ALTITUD	1400 m s. n. m.
TIPO DE VEGETECIÓN	Selva baja caducifolia
INFORMACION AMBIENTAL	Cálido y templado
SUELO	Rocoso
OTROS DATOS	------
FORMA BIOLOGICA	Herbáceo
TAMAÑO	1.5 m
ABUNDANCIA	Abundante
FRUTO	Sin fruto
FLOR	inflorescencia formada por flores sésiles
NOMBRE LOCAL	Pápalo
DETERMINO	INAH
COLECTO	Ana Karen Villa Merlán
ASOCIADA	------
OBSERVACIONES	Muy olorosa al estrujarse
USOS	Las hojas son comestibles como verdura y se usa en la medicina tradicional.
NO. DE COLECTA	01
FECHA	11/11/16

Anexo 2

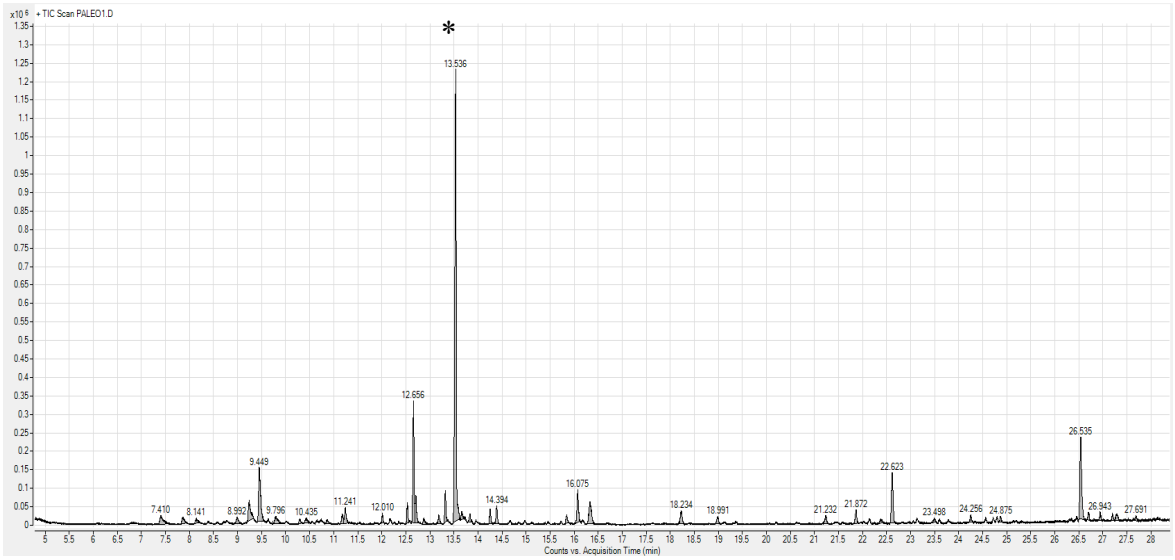
Identificación de *Porophyllum tagetoides*

Ficha descriptiva	
NOMBRE CIENTIFICO	*Porophyllum tagetoides*
FAMILIA	Asterácea
LOCALIDAD	Chilapa de Álvarez
LATITUD	17.6594
LONGITUD	-99.8672
ALTITUD	1400 m s. n. m.
TIPO DE VEGETECIÓN	Selva baja caducifolia
INFORMACION AMBIENTAL	Cálido y templado
SUELO	Rocoso
OTROS DATOS	----------
FORMA BIOLOGICA	Herbáceo
TAMAÑO	De 25 a 50 cm de alto
ABUNDANCIA	--------
FRUTO	Sin fruto
FLOR	-------
NOMBRE LOCAL	Pipisa
DETERMINO	INAH
COLECTO	Ana Karen Villa Merlán
ASOCIADA	------
OBSERVACIONES	Muy olorosa al estrujarse
USOS	Las hojas son comestibles como verdura y se usa en la medicina tradicional.
NO. DE COLECTA	01
FECHA	08/04/17

Anexo 3

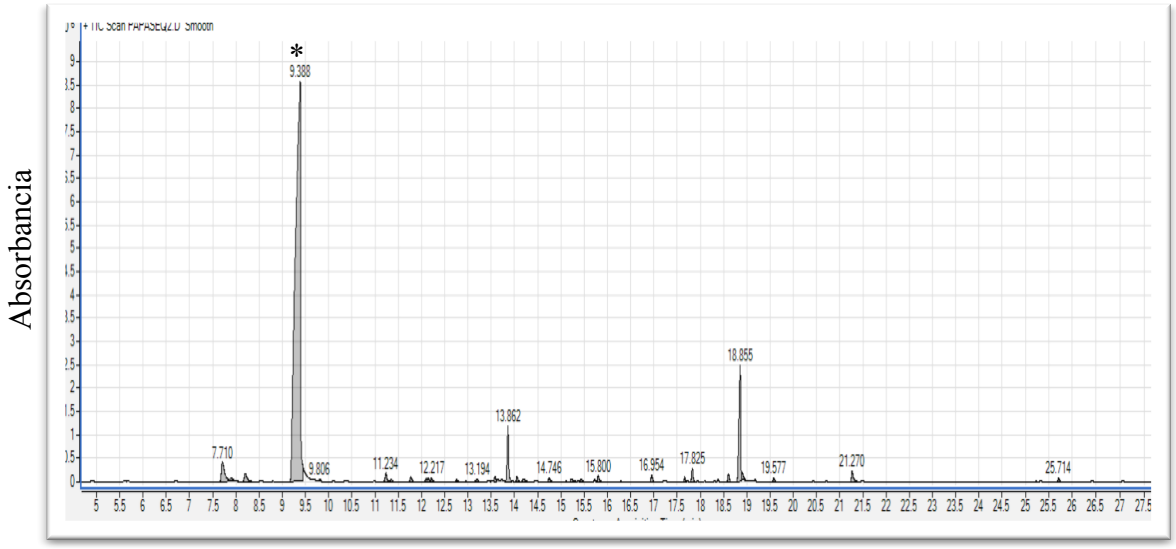
Cromatogramas.

Porophyllum ruderale subp *macrocephalum (Leaves fresh)*



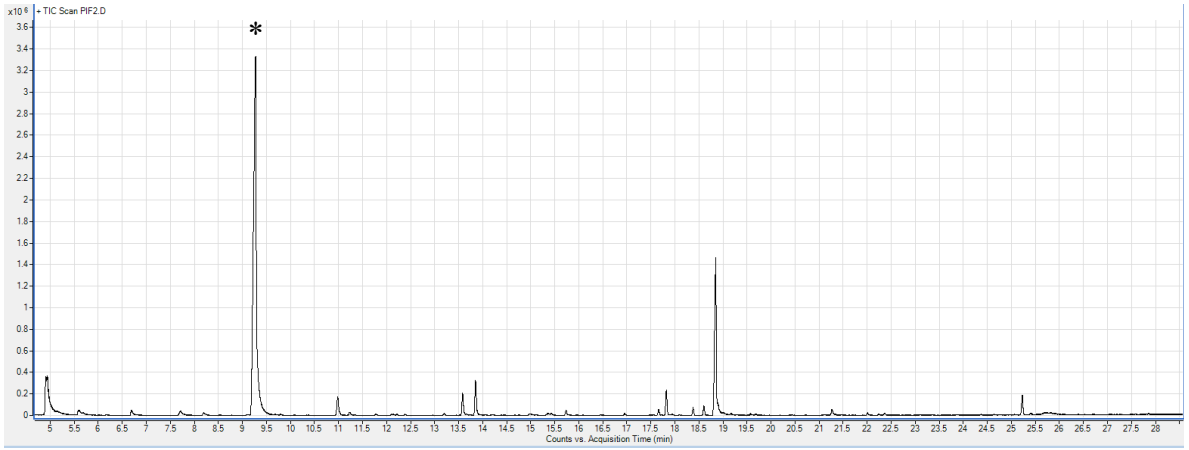
Cromatograma de gases de aceites esenciales obtenidos de hojas frescas de *Porophyllum ruderale* subp *macrocephalum*. Se analizaron los aceites esenciales obtenidos de hojas frescas de pápalo con diclorometano a una concentración (1:1000) *= Compuesto mayoritario.

Porophyllum ruderale subp *macrocephalum (Leaves dry)*



Cromatograma de gases de aceites esenciales obtenidos de hojas secas de *Porophyllum ruderale* subp *macrocephalum*. Se analizaron los aceites esenciales obtenidos de hojas frescas de pápalo con diclorometano a una concentración (1:1000) *= Compuesto mayoritario.

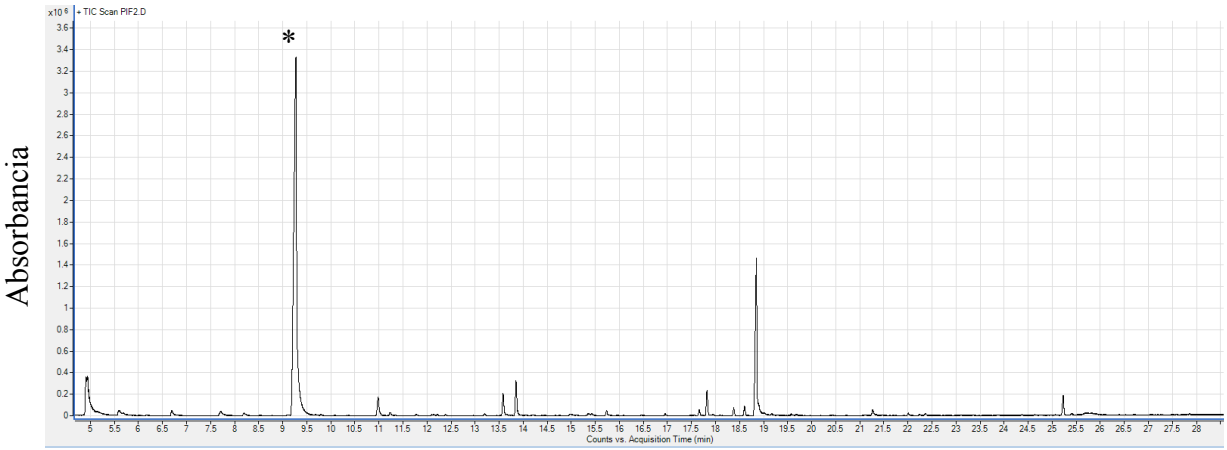
Porophyllum tagetoides (Leaves fresh)



Tiempo de retención (TR) = min

Cromatograma de gases de aceites esenciales obtenidos de hojas frescas de *Porophyllum tagetoides*. Se analizaron los aceites esenciales obtenidos de hojas frescas de pápalo con diclorometano a una concentración (1:1000) *= Compuesto mayoritario.

Porophyllum tagetoides (Leaves dry)



Tiempo de retención (TR) = min

Cromatograma de gases de aceites esenciales obtenidos de hojas secas de *Porophyllum tagetoides*. Se analizaron los aceites esenciales obtenidos de hojas frescas de pápalo con diclorometano a una concentración (1:1000) *= Compuesto mayoritario.

Anexo 4

UNIVERSIDAD AUTONOMA DE GUERRERO
ESCUELA DE CIENCIAS QUIMICO BIOLOGICAS
Laboratorio de Biomedicina Molecular
Av. Lázaro Cárdenas S/N. Chilpancingo, Gro. 39089 Tel/FAX (747)10901

Chilpancingo, Gro. _____ de _____del 20_____.
FOLIO: _____
HORA: _____

CONSENTIMIENTO INFORMADO

PROYECTO: COMPOSICIÓN QUÍMICA, ACTIVIDAD ANTIOXIDANTE Y ANTIGENOTOXICA DE ACEITES ESENCIALES DE *Porophyllum ruderale* Y *Porophyllum tagetoides*.

Nombre del participante: _____

Mediante esta carta doy mi consentimiento para participar en el estudio de investigación, entendiendo la información que se me dio previamente y aclarando todas mis dudas sobre el manejo de mi muestra.

La aceptación de la participación voluntaria se realiza firmando esta carta informada, de igual manera doy el consentimiento para que me entreviste como parte del proyecto de investigación al cual estoy participando, las preguntas constan de aspectos personales, familiares y de salud, entendiendo que la entrevista aportara información útil para interpretar resultados que se obtengan del proyecto de investigación.

A su vez, al firmar el consentimiento me estoy comprometiendo a estar disponible las veces que se me requiera otra toma de muestra, para la continuidad del proyecto. La información obtenida en este estudio, utilizada para la identificación de cada paciente, será mantenida en confidencialidad por el investigador, así como la información dada.

Por lo antes dicho prosigo a firmar el documento, mis preguntas han sido respondidas de manera satisfactoria, entiendo que los datos obtenidos en el estudio pueden ser publicados o difundidos con fines científicos. Convengo en participar en este estudio de investigación.

_____ _____

Nombre Firma del participante Nombre y Firma del Investigador

UNIVERSIDAD AUTONOMA DE GUERRERO
ESCUELA DE CIENCIAS QUIMICO BIOLOGICAS
Laboratorio de Biomedicina Molecular
Av. Lázaro Cárdenas S/N. Chilpancingo, Gro. 39089 Tel/FAX (747)10901

Encuesta para el participante.

Chilpancingo, Gro. _____ de _____del 20_____.
FOLIO: _____

Nombre del participante: _____
Edad: _____ Sexo: _____ Lugar de origen: _____
Dirección: _____
_____Localidad/ Municipio _____
_____ C.P: _____ Tel: _____
Ocupación: _____.

A qué hora ingirió su ultimo alimento: _____

1. ¿Cómo considera su estado de salud?

a) Bueno
b) Regular
c) Malo

2. ¿Realiza algún deporte?

a) Si Cual _____
b) No

3. ¿Con que frecuencia hace ejercicio?

a) Todos los días
b) 1 vez por semana
c) 2 veces al mes
d) Nunca

4. ¿Actualmente está consumiendo algún suplemento alimenticio?

a) Si Cual _____
b) No

5. ¿Consumes bebidas alcohólicas?

a) Si
b) No

6. ¿Con que frecuencia?

a) Siempre
b) Algunas Veces
c) Nunca

7. ¿Usted fuma?

a) Si
b) No

8. Ingiere algún tipo de droga

a) Si ¿Hace cuánto tiempo? _____
b) No

9. ¿Se ha sometido a Rayos X?

a) Si ¿Hace cuánto tiempo? _____
b) No

10. ¿Ha estado en tratamiento con quimioterapia?

a) Si ¿Hace cuánto tiempo? _____
b) No

11. ¿Ha estado expuesto a solventes?
(Tinner, acetona, metanol, isopropanol, xileno, tolueno)

a) Si ¿Hace cuánto tiempo? _____
b) No

12. ¿Realiza actividades expuesto al sol por largo tiempo?

a) Si ¿Hace cuánto tiempo? _____
b) No

13. ¿Hace cuánto tiempo se desparasito? _____

a) Si ¿Qué tratamiento utilizase? _____
b) No

14. ¿tiene o ha tenido algún familiar (hijo, sobrino o hermano) con alguna malformación (¿LPH, espina bífida, falta de alguna parte de su cuerpo?

a) Si ¿Hace cuánto tiempo? _____
b) No

15. ¿Padece o sabe que tiene alguna de las siguientes enfermedades actualmente?

a) Cáncer

b) Anemia
c) Hepatitis
d) Diabetes
e) Artritis
f) Resequedad excesiva de su nariz
g) Alteraciones en sus riñones
h) Enfermedades infecciones agudas (2 semanas antes del muestreo)

Le agradecemos su participación y nos comprometemos a que la información recabada es de suma cooficialidad y por lo tanto no podrá ser usado con otros fines que no sean para efecto de la investigación planteada. Su nombre jamás aparecerá en ningún informe.

Término de la entrevista _____