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IDENTIFICATION OF VOLATILE CONSTITUENTS *OF* ALBAHA *PLUECHEA OVALIS* AND THEIR POTENT MANIFOLD BIOLOGICAL ACTIVITIES

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ABSTRACT

Cancer and microbial infections are main causes of morbidity and mortality all over the world. *Pluchea* is a genus of flowering plants belongs to *Asteraceae* (*compositeae*) family, *Pluchea ovalis* (PO) is one of its species growing in Albaha region. The volatile constituents were extracted with pet. ether and were identified using GC/MS analysis. It was found that, the volatile constituents consist of a mixture of 15 compounds in which □- selinene, humulene oxide and β- eudesmol constitutes about 61%. The aerial parts of PO were extracted with pet ether. The pet ether extract was fractionated into unsaponifiable fraction and fatty acids and their constituents were identified by GC/MS analysis. In the present study Gram (+) bacteria such as *Staphylococcus aureus*, Bacillus *subtilis* and Gram (-) bacteria such as *Escherichia coli*, *Pseudomonas aeuroginosa* have been used. In addition; fungi such as *Aspergillus flavus* and *Candida albicans* were also studied. The results of antimicrobial activity of different extracts demonstrated that, pet. ether extract exhibited highest activity against *B. subtilis*, *E. coli* and *S. aureus* with MIC=6.4, 10.0 and 7.2 mg/ml respectively. The results of antitumor activity against colon carcinoma cell line (HCT-116) showed that alcoholic extract is most active extract with IC_{50} = 21.4 ± 1.3 µg/ml.

KEYWORDS: *Pluchea ovalis*, Volatile constituents, Lipid fraction, Antimicrobial, Antitumor activity.

INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality all over the world. Chemotherapy is routinely used for cancer treatment. Alternative treatments have also been advocated for cancers, nevertheless loads of which are based on medicinal plants and their extracts. The anticancer properties of various plants were known from ancient. Medicinal plants are still widely been used in production of new drugs and are a reservoir of natural chemicals which can be useful against cancer.[1-2]

The genus *Pluchea* is distributed worldwide in tropical and subtropical regions of Northern and Southern America, Africa, Asia, and Australia and comprises of about 80 species.[3-4] The plants of *Pluchea* genus have been utilized in traditional medication for the treatment of many illnesses like antipyretic, nerve tonic, diaphoretic (in fevers), cytotoxic, antiulcer, antipyretic, antibacterial, antifungal, antiviral, astringent, anti-inflammatory, hepatoprotective, against digestive diseases, smooth muscle relaxant, dysuria, hemorrhoids and laxative.[5-9] Previous researches on this genus demonstrated the presence of Eudesmane type sesquiterpenoids, tannins, alkaloids, triterpenoids lignan glycosides and flavonoids.[10-13] This manuscript represents the investigation of volatile constituents and isolation of lipid components of PO growing in Albaha region, KSA. Additionally, it the evaluation of different extracts for cytotoxic and antimicrobial activity has also been studied.

MATERIALS AND METHODS

Plant material

Pulchea ovalis (PO) was collected from Albaha region, KSA, in the month of June 2018. The plant was identified by Dr. Hedir Abdelkader at Biology Department, Faculty of Science, Albaha University, KSA. A voucher specimen was deposited at Biology Department, Albaha University. The above ground portion parts were dried in shade for 10 days. After complete drying, the plant was ground into fine powder for extraction.

Extrication of the volatile compounds and lipid constituents

150 g of aerial portion was extracted with pet. ether (40-60°C) in a Soxhlet for two days till exhaustion to afford a greenish color extract. This extract was divided into two portions. The first portion was passed over fuller's earth to remove the colored pigments to afford a clear yellowish extract; the solvent was evaporated using rotatory evaporator to give the pet. ether extract which was analyzed using GC/MS. The solvent of second

portion was evaporated in the same manner to give a semisolid oily residue which was saponified by refluxing with 100 ml N/2 alcoholic KOH. The alcoholic residue has been reduced to about 25 ml and diluted with cold distilled water. The unsaponifilable compounds were extracted with successive portions of chloroform (3×100ml). The combined chloroform residue was partitioned with distilled water, dehydrated over anhydrous sodium sulphate and evaporated in *vacuo* to obtain a yellowish brown semi solid residue of unsaponifiable compounds.

The hydroalcoholic soap solution after saponification was rendered acidic (pH=2) with H_2SO_4(5%). The liberated fatty acids were thoroughly extracted several times with diethylether. The mixed ether extract was partitioned with distilled water until these are without of acidity and dehydrated over anhydrous sodium sulphate. The solvent was removed in *vacuo* at about 40^0C till dryness.

The fatty acids were dissolved in 75ml dry methanol containing 4-5% dry HCl and refluxed on a boiling water bath for 4 h. The solvent was concentrated by evaporation up to 20 ml and diluted with 100 mL distilled water. The **FAMEs** were extracted with successive portions of diethylether (3×100ml), cleaned with distilled water to free from acidity, desiccated on anhydrous sodium sulfate, filtered and evaporated at 40 ^{0}C.

GC-MS analysis

The GC/MS analysis was performed at the Central Lab National Research Center, Dokki, Giza, Egypt using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For MS detection, an electron ionization system having ionization energy of 70 eV was utilized; Helium gas has been utilized as carrier gas at a steady flow of 1mL/min. The injector and MS transfer column temperature has been adjusted to 280 °C. For the volatile compounds, the oven temperature was adjusted at an initial temperature of 40 °C (hold 3 min) to 280 °C as a concluding temperature at rising rate of 5 °C /min (hold 5 min).

For the unsaponifiable fraction, the oven temperature was programmed at an initial temperature of 50 °C (hold 2 min) to150 °C at an increasing rate of 7 °C /min, then to 270 at an increasing rate 5 °C /min (hold 2min) and then to 310 as a final temperature at an increasing rate of 3.5 °C /min (hold 10 min).

While for the FAME: The oven temperature was programmed at an initial temperature 150 °C (hold 4 min) to 280 °C as a final temperature at an increasing rate of 5 °C /min (hold 4 min).

The quantification of all the identified components was investigated using a percent relative peak area and comparing their retention indices relative to C8-C26 (n-alkane series) with those already reported in the Wiley spectral library collection and NSIT library, in addition to the previous literature.[14-15]

Preparation of different plant extracts for biological evaluation

The air dried fine powder of the plant material was divided into six samples (30 g each) and macerated separately in 100 ml of following solvents: chloroform, ethyl acetate, n-butanol, methanol, water and pet ether for two days with shaking from time to time. The solution was filtered and the process was repeated three times till complete extraction and the solvents were evaporated to afford the chloroform extract (1P), ethyl acetate extract (2P), n-butanol extract (3P), methanol extract (4P), water extract (5P) and pet. ether extract (6P) respectively that have been screened for their biological activities such as antimicrobial and cytotoxicity.

Antimicrobial Activity test

The antimicrobial activity was evaluated using different organisms such as *Staphylococcus aureus* (ATCC 12600) and *Bacillus subtilis* (ATCC 6051) as Gram-positive bacteria; *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) as Gram-negative bacteria. The *Candida albicans* (ATCC 7102) and *Aspegillus flavus* (ATCC 19433) fugal strains were screened using a modified Kirby-Bauer disc diffusion method.[16] The bacterial and fungal microorganisms were acquired from laboratory collection strains of Micro Analytical Center, Faculty of Science, Cairo University. Ampicillin and Amphotericin B (Bristol-Myers Squibb, Switzerland) have been employed as standard antibacterial and antifungal drugs respectively. The test was carried out as described earlier.[17] and the MIC values were determined as explained by NCCLS.[18]

Evaluation of cytotoxicity on HCT-116 cell lines

This work was done at Al-Azhar University**,** The Regional Center for Mycology & Biotechnology. HCT-116 (colon carcinoma) cell lines were attained from VACSERA Tissue Culture Unit.

Chemicals

Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dyes have been obtained from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA obtained from Lonza.

Cell culture

The cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) complemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. Culture was kept at 37 °C in a humidified incubator (5% CO_2) and was sub-cultured after two weeks of times.

Cytotoxicity evaluation using viability assay

The test was done according to previously reported methods.[19,20] The 50% inhibitory concentration (IC_{50}), is concentration necessary to produce 50% toxicity, which can be was anticipated by graphic plots by means of dose response curve for all concentrations utilizing Graphpad Prism software (San Diego, CA. USA).

RESULTS AND DISCUSSION

The data in Table-1 and Figure-1 represented the GC-MS results of volatile components of pet. ether extract of PO. The constituents have been identified based on the matching with NIST library and its has been established that the volatile constituents contain fifteen compounds. Most of them are sesquiterpene hydrocarbons or oxygenated sesquiterpenes where sesquiterpene hydrocarbons forming about 52.85% with Selinene as a main component (33.40%) while oxygenated sesquiterpenes constitutes about 40.88% in which Humulene oxide is the most abundant one (12.42%). It has also been noted that the other classes like n-hydrocarbons, acids and esters are present in low percentage while the aromatic and heteroaromatics, mono and di terpenes are completely absent. The present data is not in agreement with that of reported data for *Pulchea*. Paini *et al.*[21] found that *Pluchea indica* essential oil contains 66 components in which (10S,11S)-Himachala-3-(12) 4-diene (17.13%) comprises the highest proportion of volatile compounds. The rest of components belong to aliphatic unsaturated hydrocarbons (1.35%), alcohols (6.16%), ketones (3.49%), heterocyclic hydrocarbons (0.05%), aldehydes (1.79%), esters (0.08%), sulfoxides (0.06%), and aromatic hydrocarbons (2.05%). Similarly, Pooja *et al.*[22] reported that essential oil of *P. lanceolata* contain oxygenated monoterpene in high percentage (53.4%) with four major compounds as: linalool (32.2%), α-terpineol (8.0%) and linalylacetate (5.6%), sesquiterpenes (20.9%), mostly composed of α-copaene (3.6%), *trans*-caryophyllene (8.5%) and oxygenated sesquiterpene, consisting of spathulenol (7.4%) and aromatic hydrocarbon composed of naphthalene, 1, 6 dimethyl-4-(1-methylethyl) (4.3%). Reham *et al.*[23] reported about the volatiles of *P. dioscoridis* where GC/MS analysis revealed the presence of 82 compounds in which four components belong to sesquiterpene hydrocarbons ($C_{15}H_{24}$) and their percentages ranged from 7.68% to 1.25%. The outcome of GC-MS study of volatiles obtained from *P. ovalis* growing at Albaha region are remarkably different from the one previously reported.

The GC-MS screening of unsaponifiable fraction (Fig. 2 and Table 2) showed that it contain twenty one compounds in which butyl hydroxy toluene constitute 16.82%, sesquiterpenes about 26.17% with á-Eudesmol as a main compound, amides constitutes about 25.32% with Hexadecanamide ($C_{16}H_{33}NO$) forming 10.84% in addition of a series of hydrocarbons (12.40%) with one steroidal compound identified as 3-ethyl-3-hydroxy-17-Androstanone (1.9%).

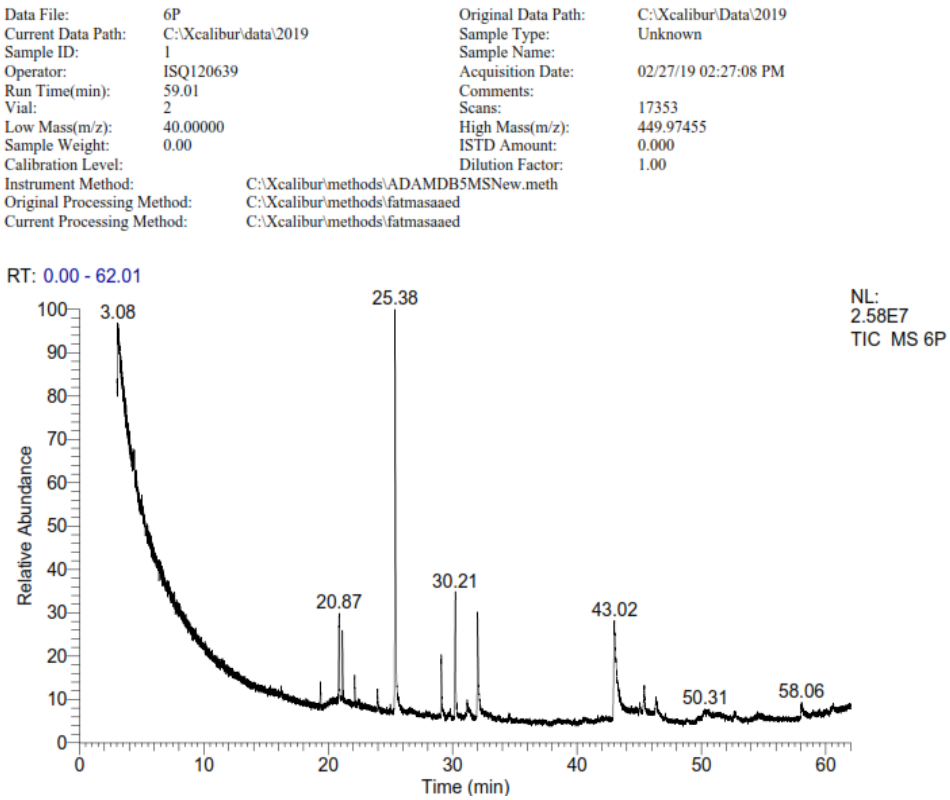

Fig. 1: GC chromatogram of the volatile constituents of PO.

Library Search Report

NRC-GC/EI-MS Lab			
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Comments:		Run Time(min):	53.02
Scans:	15592	Vial:	3
High Mass(m/z):	700.02954	Low Mass(m/z):	40.00000
ISTD Amount:	0.000	Sample Weight:	0.00
Dilution Factor:	1.00	Calibration Level:	
Instrument Method:	C:\Xcalibur\methods\sameh\unsap.meth		
Original Processing Method:	C:\Xcalibur\methods\sameh\mona-un s		
Current Processing Method:	C:\Xcalibur\methods\sameh\mona-un s		

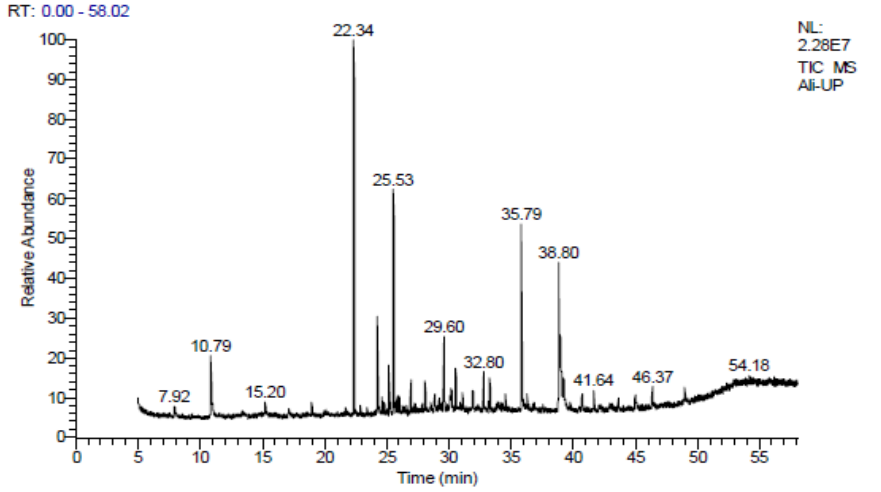

Fig. 2: GC chromatogram of the unsaponifiable matter of PO.

NRC-GC/EI-MS Lab			
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Sample Name:		Sample ID:	1
Acquisition Date:	12/05/19 09:32:18 AM	Operator:	ISQ120602
Comments:		Run Time(min):	36.49
Scans:	10732	Vial:	1
High Mass(m/z):	649.99304	Low Mass(m/z):	40.00000
ISTD Amount:	0.000	Sample Weight:	0.00
Dilution Factor:	1.00	Calibration Level:	
Instrument Method:	C:\Xcalibur\methods\sameh\Sap.meth		
Original Processing Method:	C:\Xcalibur\methods\sameh\mona-un s		
Current Processing Method:	C:\Xcalibur\methods\sameh\mona-un s		

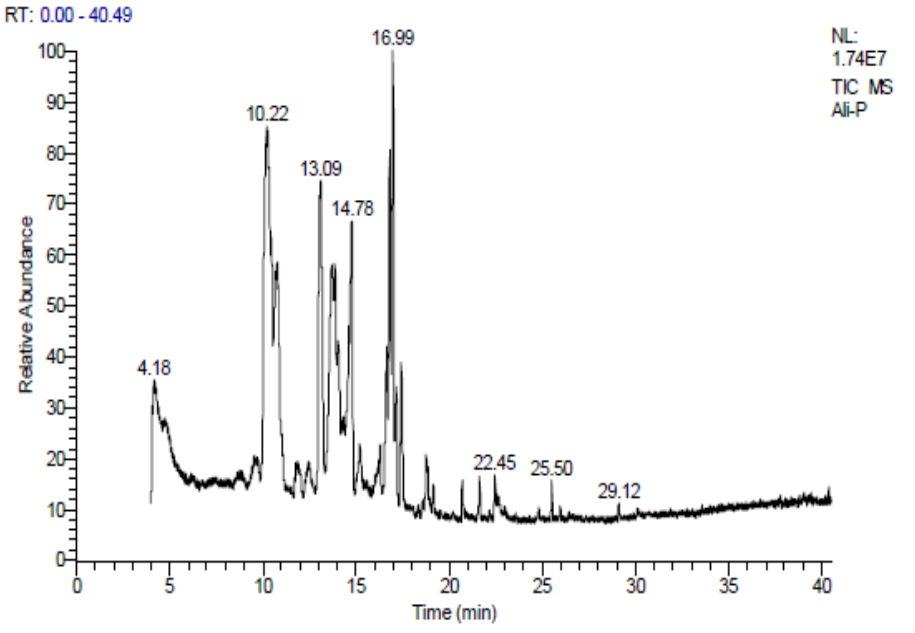

Fig. 3: GC chromatogram of FAME of PO.

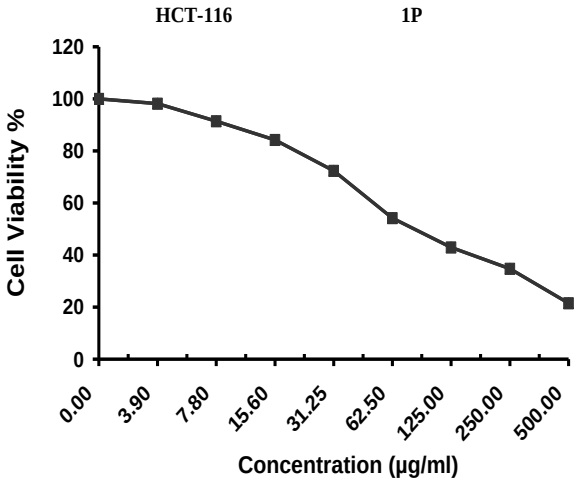

Fig. 4: Inhibition of HCT-116 cells by chloform extracts (1P).

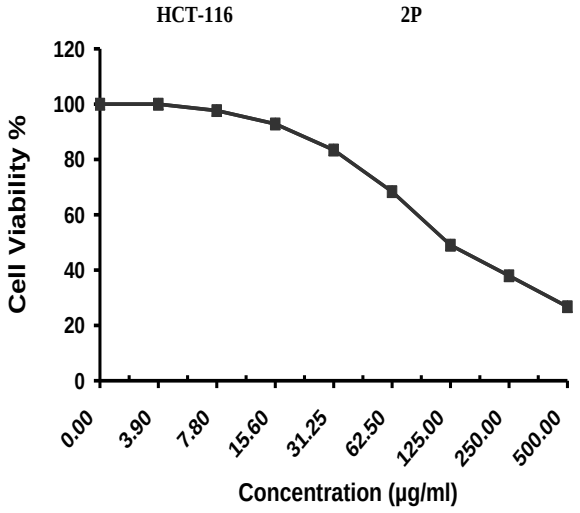

Fig. 5: Inhibition of HCT-116 cells by ethyl acetate extracts (2P).

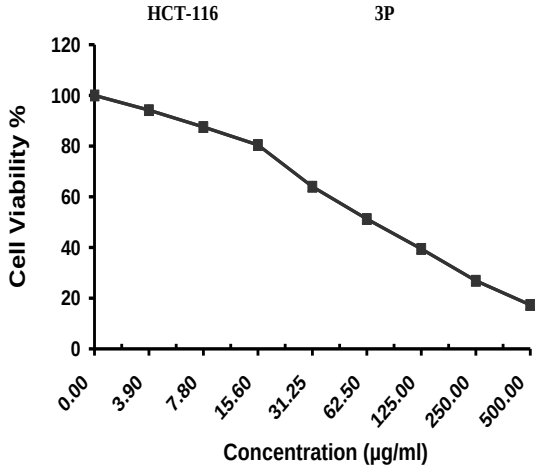

Fig. 6: Inhibition of HCT-116 cells by butanol extracts (3P).

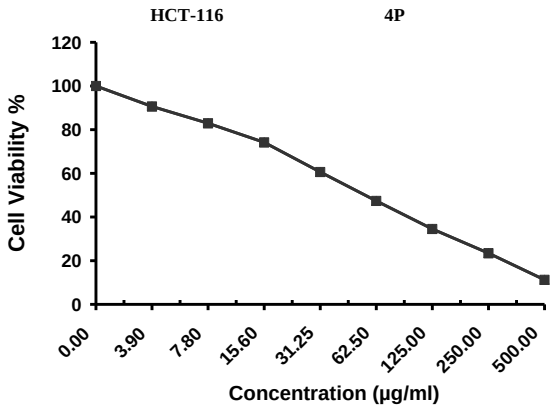

Fig. 7: Inhibition of HCT-116 cells by alcoholic extracts (4P).

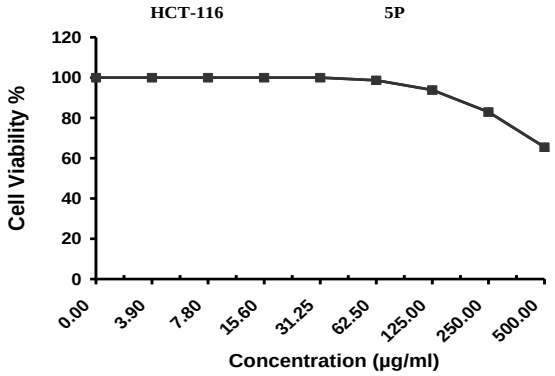

Fig. 8: Inhibition of HCT-116 cells by water extracts (5P).

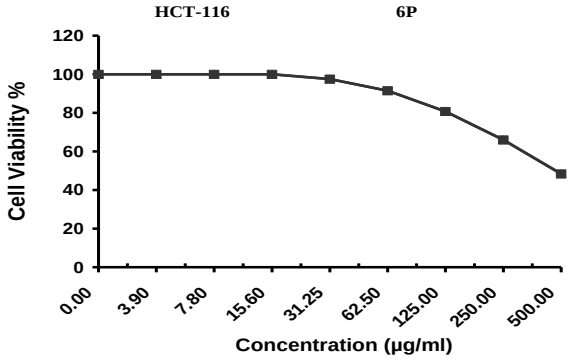

Fig. 9: Inhibition of HCT-116 cells by pet. ether extracts (6P).

Table 1: GC/MS data of the volatile constituents from PO.

Peak no.	Rt (min)	Area (%)	Mass data			Molecular formula	Compound
			M^+	bp	Fragments (%)		
1	19.38	2.74	204	105	189(35),147(85), 41(72)	$C_{15}H_{24}$	à-Guaiene
2	20.87	6.94	204	204	161(92), 105(89), 91(82)	$C_{15}H_{24}$	à-Gurjunene
3	21.14	5.44	204	162	189(22),147(55), 119(45)	$C_{15}H_{24}$	Berkheyaradulene
4	22.13	2.57	204	91	189(36),147(55), 107(84)	$C_{15}H_{24}$	Azulene,
5	23.96	1.76	204	93	147(35), 121(29), 41(52)	$C_{15}H_{24}$	à-Humulene
6	24.98	0.56	238	85	121(29), 57(86), 41(93)	$C_{15}H_{26}O_2$	Geranyl isovalerate
7	25.38	33.40	204	93	189(62),107(84), 79(85)	$C_{15}H_{24}$	á-Selinene
8	29.09	5.29	220	43	177(18), 93(45), 79(76)	$C_{15}H_{24}O$	Caryophyllene oxide
9	30.21	12.42	220	43	138(46), 109(67), 67(81)	$C_{15}H_{24}O$	Humulene oxide
10	32.01	10.41	222	59	149(23), 109(31), 43(64)	$C_{15}H_{26}O$	β- Eudesmol
11	42.99	9.76	232	81	217(21), 109(65), 53(81)	$C_{15}H_{20}O_2$	Dehydrosanssurea

							lactone
12	45.41	3.00	234	43	136(41), 109(85), 81(78)	$C_{15}H_{22}O_2$	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo-[5.1.0.0(2,4)] octane
13	46.35	2.00	304	79	106(45), 91(67), 67(51)	$C_{20}H_{32}O_2$	Arachidonic acid
14	52.63	1.19	318	41	207(15), 93(64(, 67(82)	$C_{21}H_{34}O_2$	Methyl arachidonate
15	58.06	2.53	408	43	351(6),99(29), 71(59)	$C_{29}H_{60}$	Nonacosane

Classes of the volatile chemical constituents of PO

No.	Class of Compounds	%
1	n-hydrocarbons	2.53
2	Sesquiterpenes	52.85
3	Oxygenated Sesquiterpenes	40.88
4	Acids	2.0
5	Esters	1.75

Table 2: GC/MS of the unsaponifiable fraction of PO.

Peak no.	Ret. time	%	Mol.wt	Molecular formula	Compound
1	10.80	4.47	164	$C_7H_{16}O_4$	1,3,4-Trimethoxy-2-butanol
2	22.34	16.82	220	$C_{15}H_{24}O$	Butyl hydroxy toluene
3	24.23	6.30	220	$C_{15}H_{24}O$	Campherenone
4	25. 16	2.93	220	$C_{15}H_{24}O$	Caryophyllene oxide
5	25.25	1.12	220	$C_{15}H_{24}O$	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol,4,4-dimethyl
6	25. 53	10.35	222	$C_{15}H_{26}O$	á-Eudesmol
7	25.83	1.24	234	$C_{16}H_{26}O$	7,10,13-Hexadecatrienal
8	26. 00	2.75	310	$C_{22}H_{46}$	n-Docosane
9	26. 94	1.62	328	$C_{22}H_{32}O_2$	Doconexent
10	28. 07	4.69	358	$C_{21}H_{26}O_5$	Pregna-1,4-diene-3583,11,20trione, 17,21-dihydroxy
11	29. 60	4.53	344	$C_{23}H_{36}O_2$	Methyl 7,10,13,16,19-docosapent
12	30.55	2.93	318	$C_{21}H_{34}O_2$	3-ethyl-3-hydroxy-17-Androstanone
13	32.80	4.21	310	$C_{22}H_{46}$	n-Docosane
14	35. 79	10.84	255	$C_{16}H_{33}NO$	Hexadecanamide
15	38.80	8.52	281	$C_{18}H_{35}NO$	9-Octadecenamide
16	38. 99	5.96	283	$C_{18}H_{37}NO$	Octadecanamide
17	40. 71	2.76	346	$C_{19}H_{22}O_6$	Isochiapin-B
18	41. 64	3.67	390	$C_{24}H_{38}O_4$	Di-(2-ethylhexyl)phthalate
19	43. 63	1.43	408	$C_{29}H_{60}$	n-Nonacosane
20	46. 37	2.17	450	$C_{32}H_{66}$	n-Dotriacontane
21	48. 95	1.89	618	C44H90	n-Tetratetracontane

Table 3: GC/MS Data of FAME of PO.

Peak no.	Compound	R_t (min.)	Area (%)	M. wt.	Chemical formula
1	methyl 2,4,6-Hexadecatrienoate	10.22	8.85	264	$C_{17}H_{28}O_2$
2	methyl linolenoate	13.71	11.49	292	$C_{19}H_{22}O_2$
3	methyl 10,12-Octadecadienoate	14.77	16.23	294	$C_{19}H_{34}O_2$
4	methyl 6,9-Octadecadienoate	16.83	7.52	294	$C_{19}H_{34}O_2$
5	methyl elaidate	16.98	12.95	296	$C_{19}H_{36}O_2$
6	methyl 3-hydroxy Octadecanoate,	17.43	9.74	218	$C19_9H_{38}O_3$
7	methyl arachidate	18.76	5.5	326	$C_{21}H_{42}O_2$
8	methyl behenate	22.44	2.98	354	$C_{23}H_{46}O_2$
9	methyl lignocerate	25.50	3.25	382	$C_{25}H_{50}O_2$
10	Unknowns	21.22			

Table 4: Antimicrobial activity of different extracts of PO.

Sample		Inhibition zone diameter (mm/mg Sample)					
		Bacterial species				Fungi	
		G+		G-			
		B. subtilis	*St. aureus*	*E. coli*	*Ps.aeruginosa*	*A. flavus*	*C. albicans*
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0
Standard	Ampicillin Antibactr. Agent	31	24	30	28	--	--
	Amphotericin B Antifungal agent	--	--	--	--	16	21
1P		23	21	21	19	0.0	0.0
2P		22	23	23	21	0.0	0.0
3P		20	21	19	20	0.0	0.0
4P		15	14	14	12	0.0	10
5P		13	12	11	11	0.0	0.0
6P		26	25	26	21	0.0	10

Table 5: Determination of MIC of PO active extracts.

Sample	MIC (mg/ml)				
	G^+		G^+		Fungi
	B. subtilis	*St. aureus*	*E.coli*	*Ps. aeruginosa*	*C. albicans*
2P	**--**	**--**	**--**	**13**	**--**
6P	**6.4**	**10**	**7.2**	**--**	**58**

Table 6: Cytotoxicity of different extracts of PO.

Sample conc. (µg/ml)	1P		2P		3P		4P		5P		6P	
	Viab.%	Inhib. %	Viab.%	Inhib. %	Viab. %	Inhib. %	Viab. %	Inhib. %	Viab. %	Inhib. %	Viab. %	Inhib. %
500	21.49	78.51	26.78	73.22	17.35	82.65	11.27	88.73	65.47	34.53	48.36	51.64
250	34.76	65.24	37.94	62.06	26.89	73.11	23.46	76.54	82.91	17.09	65.97	34.03
125	42.93	57.07	49.02	50.98	39.47	60.53	34.52	65.48	93.84	6.16	80.73	19.27
62.5	54.20	45.8	68.36	31.64	51.23	48.77	47.38	52.62	98.65	1.35	91.48	8.52
31.25	72.38	27.62	83.44	16.56	63.97	36.03	60.59	39.41	100	0	97.52	2.48
15.6	84.19	15.81	92.83	7.17	80.42	19.58	74.21	25.79	100	0	100	0
7.8	91.42	8.58	97.65	2.35	87.56	12.44	82.94	17.06	100	0	100	0
3.9	98.16	1.84	100	0	94.21	5.79	90.63	9.37	100	0	100	0
0	100	0	100	0	100	0	100	0	100	0	100	0

The results of GC/MS of FAME of PO were shown in Fig.3 and Table 3. It proved the presence of nine fatty acids; five of them are unsaturated representing about 57.04% and four compounds of saturated fatty acids forming about 21.74%. The last 21.22% are unknown ones. The unsaturated fatty acids are classified into three categories, the fist one is mono unsaturated FA (12.95%), the second is desaturated FA (23.75%) with methyl 10,12-Octadecadienoate as major (16.23%) component and the third one is poly unsaturated FA with methyl linolenate(11.49%) as the main acid, while methyl 3-hydroxy Octadecanoate constitutes the most abundant one in saturated FA(9.74%). These data were in accordance with that reported by Hidayat *et al.*[8]

Antimicrobial test of PO extracts was conducted against six microorganisms using the agar disc diffusion assay and the results are shown in Table 4 and table 5. The activity can be classified into four levels: weak activity (IZ zone =9-12mm), moderate activity (IZ zone =13-15mm) strong activity (IZ zone =16-21mm), and highly active (IZ more than 21mm). The data of the inhibition zones (mm) of various microorganisms showed that, the pet. ether extract of PO (6P) exhibited the highest antimicrobial activity against all the selected strains especially in case of *E. coli* (IZ=26mm, MIC=72mg/ml,), and moderate activity against the yeast *C. albicans* (IZ=10mm, MIC=58mg/ml). All the extracts depicted no toxicity against fungus *A. flavus*. The chloroform, ethyl acetate and butanol extracts (1P, 2P and 3P) showed high activity against all tested bacteria (IZ=19-23mm). The activity of methanol and water extracts (4P and 5P) ranged from weak to moderate (IZ-11-15mm).So, our findings are partially in agreement with that reported by Rawinipa and Suchanya.[24] They evaluated antimicrobial action of extract of fresh and dry flowers, leaves, and roots of *P. indica* and they found that, the young roots possess potent toxicity against

Bacillus cereus, *Pseudomonas fluorescens* and *Salmonella typhimurium* (0.16, 0.16 and 0.32 mg/mL MICs, respectively). However, the inhibitory action against *E. coli* was very weak. Also, Mandeel and Taha.[25] reported that the *in vitro* antifungal activity of the aqueous, ethanol, chloroform, petroleum ether residues of *P. ovalis* was evaluated and the results proved that all the extracts exhibit inhibitory effect against all tested strains.

The cytotoxic activity of different extracts of PO against HCT-116 (colon carcinoma) cell line demonstrated varied inhibitory effects. The data presented in Table 6 and Figures 4-9 proved that the alcoholic extract (4P) and the butanol extract (3P) exhibit the highest activity of 88.73% and 82.63% inhibition of the colon carcinoma cells with IC_{50} = 56.3± 2.3 µg/ml and 69 ± 3.8 µg/ml respectively. Also the results proved that the water and pet. ether extracts (5P and 6P) displayed weak inhibitory activity against colon carcinoma cells with IC_{50}. All of them = > 500 µg/ml and 477± 9.7 µg/ml respectively. The strong activity of the alcoholic extract might be occurrence of polyphenols and some of the flavonoids which found to be present during the phytochemical screening. These compounds have potent antioxidant and anticancer activities as reported by Czaplinska *et al*.[26] These results are in agreement with that of reported by Soumita *et al*.[27] They demonstrated that *Pluchea indica* extract notably hinder sarcoma-180 cell growth in a dose dependent mode in *in vivo* mice model. Gridling et al.[11] tested the methylene chloride extract of *P. odorata* on MCF-7 breast cancer cells and found that it can inhibit cell proliferation by 90% at a concentration of 4mg/ml. Moreover, it was found that, *P. odorata* contains flavonoids, triterpenes, phytosterols and a eudesmane-type sesquiterpene.[28-29] However; Monks *et al*.[30] reported that the *P. sagittalis* residues show potent toxicity against the HT29 human colon adenocarcinoma cells and NCI-H460 human lung cancer cells.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval: The study does not involve the use of humans or animals, hence does not require ethical approval.

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