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Chemical composition, antioxidant, cytotoxic and antimicrobial activities of *Pimenta racemosa* (Mill.) J.W. Moore flower essential oil

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Abstract
Hydrodistilled essential oil obtained from *Pimenta racemosa* fresh flower was analyzed by GLC-MS. Fifty two components were identified, including 3.14% monoterpenes, 94.85% oxygenated monoterpenes and 0.42% oxygenated sesquiterpenes where 1,8-Cineole (75.4%) and linalool (9.08%) represented the major constituents. Quantification of the oil constituents was carried out using GLC-FID analysis where 1,8-cineole was detected at 428.3 µg/ml. The oil exhibited potential antioxidant activity when tested by DPPH radical scavenging assay where SC_{50}=5.95 µg. The cytotoxic activity was evaluated using MTT assay compared with 1,8-cineole and doxorubicin. The oil showed significant activity against human carcinoma cell lines including colon (HCT-116), hepatic (HepG-2), lung (A-549) and intestinal epithelium (Caco-2) with IC_{50} values 1.71, 3.67, 1.35, and 2.63 µg, respectively. The oil cytotoxicity exceeded 1,8-cineole except for HepG-2. Additionally the antimicrobial activity was evaluated using agar disc diffusion and MIC where the oil exhibited the best activity against *Bacillus subtilis* and *Geotrichum candidum* with MIC=0.49 and 0.98 µg, respectively

Keywords: *Pimenta racemosa*, essential oil, antioxidant, cytotoxic, antimicrobial

1. Introduction
Essential oils are widely used for antimicrobial, antiparasitical, insecticidal and other medicinal activities. Additionally, they are used as flavour in cosmetics, pharmaceuticals and food industries [1]. *Pimenta* is a group of trees native throughout the Caribbean region. *P. racemosa* J.W. Moore is known as the "bay tree" and it has been naturalized to different African and Asian countries for its essential oil (EO) [2, 3]. Eugenol [4 -6] and 1,8-cineole [7, 8] were the main constituents identified in *P. racemosa* leaf EO which was recognized for its antioxidant [6, 9-11], antimicrobial [12-18], cytotoxic, virucidal [19], mosquito larvicidal [20], dysmenorrheal [21], insecticidal [22], nematicidal [23] and fumigant [24] activities.
Although EO of *P. racemosa* leaf had attracted the researchers for many phytochemical and biological studies, there is only one report for flower EO composition and nothing was reported for its biological activities where Aboutabl *et al*., [25] stated that eugenol and isoeugenol were the major components of leaf and flower EO in *P. racemosa* cultivated in Egypt.
The authors studied phenolic profile, anti-inflammatory, antinociceptive, anti-ulcerogenic and hepatoprotective activities of the leaves (under publication). In continuation of our work, we aim to investigate the EO composition of *P. racemosa* flower cultivated in Egypt, in addition to its antioxidant, antimicrobial and cytotoxic activities against different human cell lines.

2. Materials and methods
2.1. Plant material
Flowers of *P. racemosa* (Mill.) J.W. Moore (syn. *Myrtus caryophyllata*, Lacq. Not L, *P. acris* Kostel) were collected from Zohreya garden, Giza, Egypt during the flowering stage in September 2014. Voucher specimen has been deposited at Orman botanical garden (00099CP @ 04-07-04-01). Identification was done by Dr. Trease Labib, former head specialist of plant taxonomy at Orman botanical garden, Giza, Egypt which is matching with the previous report for identification of this plant [26].

2.2. Preparation of essential oil
EO was obtained by hydro-distillation of fresh *P. racemosa* flowers (320 g) for 6 hours using a Clevenger-type apparatus. It was dried over anhydrous sodium sulphate and kept in sealed vial at -2 °C for analyses.

2.3. Gas liquid chromatography-mass spectrometry (GLC-MS)

1 µl of EO (dissolved in *n*-hexane, 100 µl/ml) was injected into a Shimadzu GC/MS-QP5050A (Tokyo, Japan) equipped with PAS-5 ms capillary column (30 m x 0.32 mm; 0.25 µm film thickness) with splitless injector and directly coupled to Shimadzu MS-QP5050A quadrupole mass spectrometer. The injector temperature was 250°C while initial temperature was 45°C isothermal for 3 min and raised to 280°C with rate 8 °C/min and 10 min isothermal. Helium was used as carrier gas (1 ml/min). The mass spectrophotometry detector is in electron impact ionization mode and ionizing energy was 70 eV. The ion source temperature was 230°C. Kovats indices (KI) were calculated with respect to a set of co-injected standard hydrocarbons mixture (C_8-C_{24}).

2.4. Gas liquid chromatography-flame ionization detector analysis (GLC-FID)

Quantitative determination of major components of investigated EO was carried out using GLC-FID analysis by injection of 1 µl (dissolved in dichloromethane, 100 µl/ml) into Trace GC Ultra (Italy) equipped with TR-WAXMS column (30 m x 0.25 mm; 0.25 µm film thickness) with splitless injector. The temperature program was started at 50 °C isothermal for 2 min and raised to 260°C at 8°C/min, 5 min isothermal. Helium was used as carrier gas (1.5 ml/min). The injector temperature was 250°C while detector temperature was 280°C. The integration was carried out using Chrom-Card software. The identification was based upon comparison of retention time of the sample peaks with available authentics. Calibration curve was carried out using serial dilution of 1,8-cineole (0.00005-0.009 µg/µl in dichloromethane).

2.5. Antioxidant activity using DPPH radical scavenging capacity

The antioxidant activity of the EO was evaluated based upon the scavenging capacity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). 0.1 ml of EO in different concentrations (5-80 µg/ml methanol) was added to 3 ml of 0.004% methanol solution of DPPH. Absorbance was measured at 517 nm after 30 min and ascorbic acid was treated similarly [27] and the scavenging activity was calculated by the following formula:

Scavenging activity (%) = $(A_0\text{-}A_1/A_0) \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the oil or ascorbic acid. SC_{50} (the amount of oil needed to scavenge 50% of DPPH radicals) was determined from the graph of percentage of inhibition plotted against the concentration of the oil.

2.6. *In vitro* cytotoxic activity

EO, authentic sample of 1,8-cineole and doxorubicin as a positive control (0-50 µg/ml) were evaluated for their cytotoxic activity against different human carcinoma cell lines including colon (HCT-116), hepatic (HepG-2), lung (A-549) and intestinal epithelium (Caco-2) using MTT assay [28, 29]. The optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells.

The percentage of viability = $[1\text{-}(OD_t/OD_c)]$ x100%

OD_t is the mean optical density of wells treated with the tested oil; OD_c is the mean optical density of untreated cells. The relation between cell viability percentage and concentration was plotted to get the survival curve for each tumor cell line. The 50% inhibitory concentration (IC_{50}) was estimated from graphic plots of the dose response curve for each concentration.

2.7. Evaluation of antimicrobial activity

The antimicrobial activity was evaluated using agar disc diffusion technique [30]. The used microorganisms were *Staphylococcus aureus* (RCMB 010028) and *Bacillus subtilis* (RCMB 010067) as Gram positive bacteria while *Escherichia coli* (RCMB 010052) and *Pseudomonas aeruginosa* (RCMB 010043) represented Gram negative bacteria. The tested fungi were *Aspergillus fumigatus* (RCMB 02568), *Penicillium italicum* (RCMB 03924) and *Geotrichum candidum* (RCMB 05097). All organisms were obtained from the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The oil was dissolved in methanol at concentration of 0.2 mg/ml. Aliquot of 100 µl was spotted on filter paper disc, air dried and deposited on the surface of inoculated agar plates. The plates were kept at low temperature before incubation, which favours the diffusion over microbial growth.

Experiments were performed in triplicate and diameters of inhibition zones were measured after 24 h for bacteria and 48 h for fungi. The antimicrobial activity was expressed as the inhibition zone diameter (mm). Amphotericin B was used as anti-fungal, while ampicillin and gentamycin were used as anti-bacteria controls (10 µg/ml). The percentage inhibition of diameter growth was calculated as follow [31]:

$$\% \text{ of inhibition} = \frac{\text{Inhibition zone diameter of sample}}{\text{Inhibition zone diameter of standard}} \times 100$$

2.8. Evaluation of minimum inhibitory concentration (MIC)

MIC of the EO was determined using 96-well microtitre dilution method [32]. Bacterial cultures were incubated in Müller-Hinton broth (MH) overnight at 37°C and a 1:1 dilution of each culture in fresh MH broth was prepared prior to use in the micro dilution assay. Sterile water (100 µL) was pipetted into all wells of the microtitre plate, before transferring 100 µL of essential oil (0.078-10 µg/ml) in DMSO. Bacterial culture (100 µL) of an approximate inoculum size of 1.0 x 10^8 CFU/ml was added to all wells and incubated at 37°C for 24 h. After incubation, 40 µL of 0.2 mg/ml *p*-iodonitotetrazolium violet (INT) solution was added to each well and incubated at 37°C. Plates were examined after 30-60 min of incubation, where microbial growth was indicated by the presence of a reddish colour which is produced when INT, a dehydrogenase activity detecting reagent, is reduced by metabolically active microorganisms to the corresponding intensely coloured formazan. Solvent control (DMSO) and the standards were included under the same conditions. All experiments were repeated at least three times. Results are reported as means ±SD.

3. Results and discussion

3.1. Identification and quantification of oil components

Hydrodistillation of fresh flowers yielded 0.33% v/w of pale yellow oil. Identified constituents are listed in Table 1 according to the order of their elution within their classes. The identification of the oil components was based upon comparing mass spectral data and KI with Wiley Registry of Mass Spectral Data 8th Edition, NIST Mass Spectral Library (December 2005) and the available literature [4-8, 33].

Table 1: Chemical composition of essential oil isolated from *P. racemosa* flowers

No.	Compound name	KI index	Area %	Mass fragments		
				M^+	B.P	Other fragments
Monoterpene hydrocarbons						
1	Santolina triene	909	0.01	136	93	121, 108, 77, 67, 53,41
2	Artemisia triene	930	0.03	136	93	121, 108,77, 67, 53,41
3	*α*- Pinene	939	0.93	136	93	121, 105, 77, 67, 53, 41
4	Camphene	954	0.01	136	93	121, 107, 79, 67, 53, 41
5	Sabinene	975	0.01	136	93	121, 105, 77, 69, 53, 41
6	*β*- Pinene	979	0.76	136	93	121, 107, 79, 69, 53, 41
7	Myrcene	991	1.13	136	93	121, 79, 69, 53, 41
8	*p*-Mentha-1(7),8-diene	1004	0.08	136	93	121, 107, 79, 67, 53, 41
9	*P*-cymene	1025	0.08	134	119	103, 91,77,65, 51
10	*(Z)-β*-Ocimene	1037	0.01	136	93	121, 105, 79, 67, 53, 41
11	*γ*- Terpinene	1060	0.27	136	93	121, 105, 77, 65, 41
12	Terpinolene	1089	0.09	136	93	121, 105, 79, 59, 43
Oxygenated monoterpenes						
13	1,8-Cineole	1031	75.4	154	43	139, 108, 93, 84, 81, 69, 56, 41
14	*trans*-Linalool oxide	1073	0.07	170	59	155, 137, 111, 94, 68, 59, 43
15	6,7- Epoxymyrcene	1093	0.01	152	79	109, 85, 59, 41
16	Linalool	1097	9.08	154	71	136, 121, 107, 93, 41
17	Exo-fenchol	1122	0.04	154	81	121, 111, 107, 93, 69, 41
18	*cis-p*-Menth-2-en-1-ol	1122	0.04	154	43	139, 121, 111, 93, 79, 69
19	Menthe-2,8-diene-1-ol	1138	0.01	152	134	119, 109, 91, 79, 67, 43
20	*trans-p*-Menth-2-en-1-ol	1141	0.06	154	41	139, 121, 111, 92, 83, 69
21	*cis*-Pinene hydrate	1144	0.01	154	71	136, 111, 96, 69, 43
22	*trans*-Verbenol	1145	0.01	152	81	150, 135, 119, 109, 94, 67, 53, 41
23	Terpinen-4-ol	1177	2.12	154	71	136, 121, 111, 93, 71, 69, 43
24	Iso-menthol	1183	0.02	156	81	145, 134, 123, 109, 94, 67, 55, 41
25	*α*- Terpineol	1189	4.21	154	59	136, 121, 93, 81, 41, 41
26	*cis*-Piperitol	1196	0.01	154	84	139, 111, 83, 69, 55, 41
27	*trans*-Carveol	1217	0.02	152	109	137, 119, 91, 84, 69, 55, 41
28	Citronellol	1226	0.07	156	69	154, 123, 109, 93, 55, 41
29	Neral	1238	1.17	152	41	137, 119, 94, 81, 69, 53
30	Carvone	1243	0.01	150	82	135, 122, 108, 93, 54, 39
31	Geraniol	1253	0.44	150	69	139, 123, 111, 93, 53, 41
32	Geranial	1267	1.8	152	69	137, 123, 109, 84, 53, 41
33	*trans*-Ascaridol glycol	1269	0.02	170	43	143, 127, 109, 84, 81, 69, 41
34	*trans*- Sabinyl acetate	1291	0.01	194	91	178, 108, 71, 65, 43
35	Neryl acetate	1362	0.03	196	69	136, 121, 107, 93, 80, 43,41
36	Gernyl acetate	1381	0.18	196	69	136, 121, 93, 43, 41
37	Geranyl isobutanoate	1515	0.01	224	69	136, 121, 107, 93, 81, 43,41
Oxygenated sesquterpenes						
38	Hinesol	1642	0.03	222	43	204, 175, 161, 148, 109,93, 82, 69, 43
39	Allihimachalol	1662	0.06	222	43	220, 204, 189, 147, 111, 93, 43
40	Khusinol	1680	0.01	220	43	202, 187, 177, 159, 133, 105, 81, 41
41	Eudesm-7(11)-en-4-ol	1700	0.3	222	43	204, 189, 161, 109, 93, 81, 69, 43, 41
42	14-Oxy-alpha-muurolene	1769	0.02	218	93	203, 185, 175, 147, 133, 119, 105, 91, 79
Others						
43	Ethyl benzene	*	0.01	106	91	106, 77, 65, 51
44	Propyl butanoate	899	0.03	102	71	89, 43, 41
45	6-Methyl-5-hepten-2-one	986	0.02	126	43	108, 93, 69, 69, 55, 41
46	Cryptone	1186	0.04	138	43	135, 123, 110, 96, 81, 67, 41
47	2- Phenyl ethyl acetate	1258	0.001	164	104	149, 91, 78, 65, 43
48	Eugenol	1359	0.21	164	164	149, 131, 121, 103, 91, 77, 55
49	Eugenol acetate	1523	0.02	206	164	149, 131, 121, 103, 91, 77, 43
50	Phenyl ethyl isobutanoate	1394	0.04	192	104	91, 71, 43
51	Butylated hydroxytoluene	1516	0.05	220	205	189, 177, 145, 115, 105, 91, 57
52	*n*-Eicosane	2000	0.01	282	57	225, 211, 197, 183, 141, 127, 113, 99, 71
% of						
	Monoterpene hydrocarbons	3.41				
	Oxygenated monoterpenes	94.85				
	Oxygenated sesquterpenes	0.42				
	Others	0.43				
	Total identified compounds	99.11				
	Total unidentified compounds	0.89				

*Identified by mass fragment from NIST database

Fifty two compounds composing 99.11% of the whole oil were identified, which included 3.41% monoterpenes, 94.85% oxygenated monoterpenes and 0.42% oxygenated sesquiterpenes. Most of the non-identified components are present as traces with relative percentage less than 0.001%.
The main constituents of EO were 1,8-cineole (75.4%) followed by linalool (9.08%), *α*- terpineol (4.21%), terpinen-4-ol (2.12%), geranial (1.8%) and neral (1.17%). Myrcene (1.13%), *α*- pinene (0.93%), *β*- pinene (0.74 %) and other minor components were also identified.
Concentrations of major EO components identified by using GC-FID analysis and calculated according to calibration curve of 1,8-cineole, are listed in Table 2 expressed as µg/ml oil. 1, 8-cineole was chosen as external standard since it represented the major identified component in the tested oil and oxygenated monoterpenes constituted the majority of oil composition. The calibration curve of cineole exhibited high linearity with coefficient of determination (R_2) of 0.9999 at the used concentrations. Data listed in Table 2 confirmed the presence of 1,8-cineole as major component of the oil (428.30 µg/ml) followed by linalool (152.02 µg/ml) and *α*-terpineol (50.37 µg/ml).
Upon comparison between EO composition of flower in the current study and data reported by Aboutabl *et al.*, [25] for *P. racemosa* flower cultivated in Egypt, we could find chemical variation where only 27 components were identified in addition to the presence of eugenol and isoeugenol as the major components. Additionally, there are significant differences in the relative percentages of monoterpenes, oxygenated monoterpenes and sesquiterpenes. This variation can be attributed to seasonal variation.

Table 2: Quantification of major components of essential oil isolated from *P. racemosa* flowers

Compound name	Conc (µg/ml)
α- Pinene	2.68
β- Pinene	25.16
Myrcene	92.30
1,8-Cineole	428.30
Linalool	152.02
Terpinen-4-ol	27.02
α- Terpineol	50.37

Moreover, comparison between EO composition of flower in the current study and reported data for leaf, showed significant differences. For example, eugenol and isoeugenol are the majors for *P. racemosa* leaf cultivated in Egypt [25]. Eugenol and myrcene were the major constituents identified in *P. racemosa* leaf oil from Benin [5, 6] while 1,8-cineole and terpinen-4-ol were the majors in Cuba tree [7, 8]. Two reports were published for EO composition of *P. racemosa* leaves from Dominica where Mchale *et al.*, [34] stated that eugenol and chavicol were the major compounds while Tucker *et al.*, [4] declared that methyl- eugenol and eugenol were the majors. Additionally, eugenol was the predominant compound for leaf oil of Bay tree growing in India [35].

3.2. Anti-oxidant activity
The scavenging activities of the *P. racemosa* flower EO against DPPH radicals were expressed in Fig. 1. The oil showed antioxidant activity in a dose-dependent manner. The highest scavenging capacities of 85.96 and 92.4% were observed at 80 µg for *P. racemosa* EO and ascorbic acid, respectively. The oil showed a substantial antioxidant capacity with SC_{50} = 5.95 µg, which exceeded ascorbic acid (SC_{50}=14.2 µg), that may be attributed to its oxygenated hydrocarbons content and other constituents.
Leaf oil of *P. racemosa* from Benin showed higher antioxidant activity when assayed by DPPH (SC_{50}=1.3 µg/ml), which most probably attributed to the difference in oil composition [6]. 1,8-cineole, linalool, *α*-pinene and *p*-cymene showed weak DPPH scavenging activity percentage [36, 37]. So, the scavenging capacity of the oil is due to the synergistic effect of all constituents. The results suggested the use of *P. racemosa* flower oil as naturally occurring antioxidant because of concerns about the safety of synthetic antioxidants.

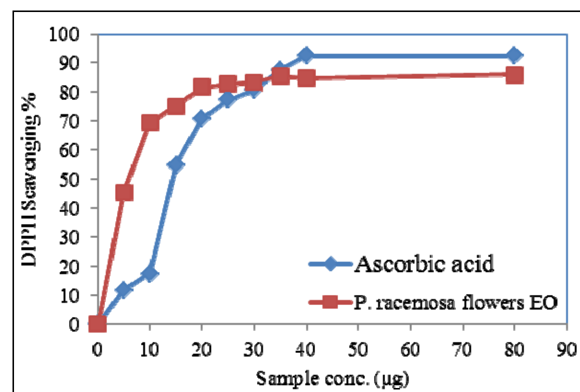

Fig 1: DPPH scavenging percentage of *P. racemosa* flower oil and ascorbic acid

3.3. Cytotoxic activity
The cytotoxic activity of tested oil, 1,8-cineole and standard doxorubicin against HCT-116, HepG-2, A-549 and Caco-2 cell lines are represented in Fig. 2, 3. IC_{50} values were used to assess the activity of the EO and 1,8- cineole against cell lines under investigation based on the protocol of the American National Cancer Institute (NCI) which recommends that crude extracts of plant origin should be considered significant for IC_{50} values ≤ 30 µg/ml as well as IC_{50} values ≤ 4 µg/ml for pure substances [38]. This evaluation is also in accordance with Srisawat *et al.* [39] where IC_{50} values were classified as follows: IC_{50} ≤ 20 µg/ml = highly active, IC_{50} 21-200 µg/ml = moderately active, IC_{50} 201-500 µg/ml = weakly active and IC_{50} > 501 µg/ml = inactive.
The EO of *P. racemosa* flower showed variable potencies against all tumor cell lines used with IC_{50} ranged from 1.35 to 3.67 µg/ml. Its cytotoxicity pattern (dose-response profile) was potential as shown by IC_{50} values against A-549 (1.35 µg/ml), HCT-116 (1.71 µg/ml), Caco-2 (2.63 µg/ml) and HepG-2 (3.67 µg/ml) cell lines. The role of major component in the EO was assessed using 1,8-cineole where it showed comparable potencies against all tumor cell lines. IC_{50} ranged from 3.02 to 5.92 µg/ml**.** 1,8-cineole cytotoxicity was significant against HepG-2 (3.02 µg/ml) followed by A-549 (3.89 µg/ml), HCT-116 (3.97 µg/ml) and to less extent against Caco-2 (5.92 µg/ml) cell lines**.** In this study the synergistic cytotoxic effect of the various oil components is clear as the cytotoxic effect of the major constituent 1,8-cineole is less than that of the EO except against HepG-2.
In vitro cytotoxic activities against liver and colon carcinoma were reported for EO of different Myrtaceae plants containing 1,8-cineole, linalool and *α*-terpineol with IC_{50} values 0.36-0.69 µg [40], which supported our results.
Terpinen-4-ol induced a significant *in vitro* cytotoxic inhibition of colorectal cells growth in a dose-dependent manner [41]. Additionally, myrcene demonstrated strong inhibitory activities against HCT-116 and Hep-G2 carcinoma (IC_{50}=1.27 and 0.93 µg, respectively) in a dose dependent manner when evaluated by MTT assay [42].

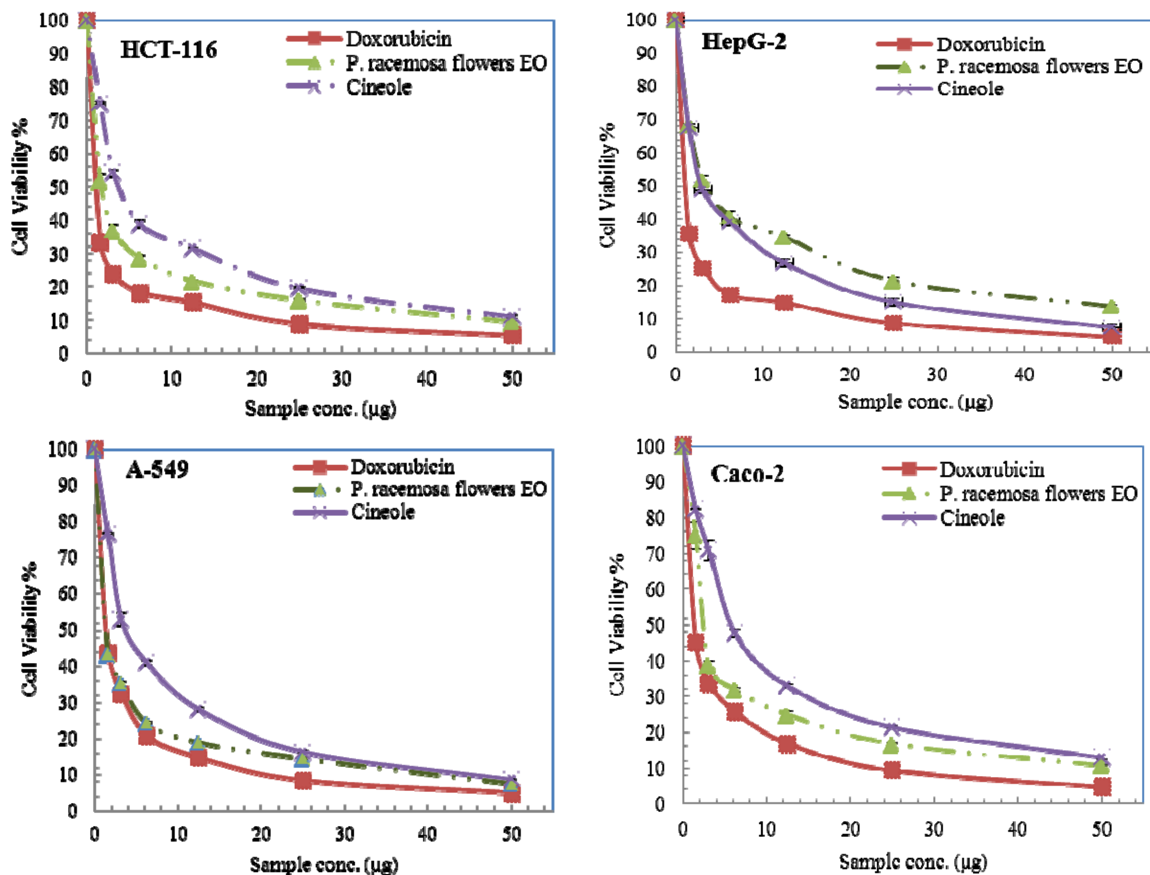

Fig 2: Effect of *P. racemosa* flower essential oil, 1,8-cineole and doxorubicin on cell viability of HCT-116, HepG-2, A-549 and Caco-2 cell lines.

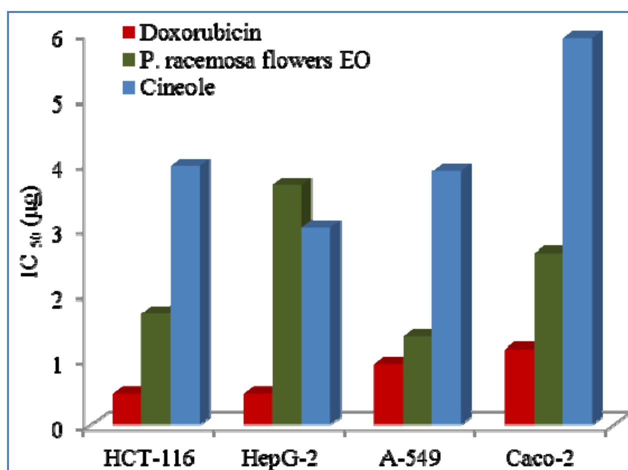

Fig 3: IC_{50} values of *P. racemosa* flower essential oil, 1,8-cineole and doxorubicin against HCT-116, HepG-2, A-549 and Caco-2 cell lines.

Geraniol (a minor component of the tested oil) was reported to inhibit 70% of cell growth of Caco-2 cell line with cells accumulating in the S transition phase of the cell cycle, and inhibition of DNA synthesis [43]. It also inhibited colon cancer cell proliferation by inducing membrane depolarisation and interfering with ionic canals and signalling pathways [1].

3.4. Antimicrobial activity

The results indicated that EO showed selective activities against different micro-organisms, especially *B. subtilis* and *G. candidum* as indicated by inhibition zone diameters, % of inhibition and MIC values. *P. aeruginosa* was resistant to the oil (Tables 3, 4).

MIC of the oil against the tested organisms ranged from 0.49 to 6.22 µg/ml. The lowest MIC values were detected against *B. subtilis* (0.49 µg) and *G. candidum* (0.98 µg), which are equal to standards ampicillin and amphotericin B, respectively that indicated the potential activity. Other significant antimicrobial activities were observed against *S. aureus* and *P. italicum* as indicated by their MIC values (0.98 and 1.95, respectively) compared with standards (0.49 and 0.98 for ampicillin and amphotericin B, respectively).

MIC is generally considered more accurate than disc diffusion assay for EO. The limitation of the oils' activity can be attributed to the low water solubility of the oil and its components, which limits their diffusion into the agar medium. Only the more water-soluble components can diffuse into the medium. The hydrocarbon components may remain on the surface of the medium or evaporate [44].

Generally, the antimicrobial activity of EO is mostly due to the presence of phenols, aldehydes and alcohols [45]. Moreover, as typical lipophiles, oils can penetrate the cell wall and cytoplasmic membrane causing disruption in the structure of polysaccharides, fatty acids and phospholipids and permeabilize them, causing loss of ions, and reduction of membrane potential leading to depletion of the ATP pool [46] in addition to cytoplasm coagulation [47] leading to cell death by apoptosis and necrosis [48]. Most of EO components identified in this study were reported to have antimicrobial action. 1,8-Cineole, terpinen-4-ol, linalool and *α*-terpineol exhibited significant inhibition of *S. aureus* and *E. coli* [49, 50]. Also, 1,8-cineole showed fungicidal activity against *A. flavus*

and *A. parasiticus* [51]. The different antibacterial activity of the EO, compared with those of their major components, can be attributed to the synergistic effect of the different components in the oil and/or by the presence of other active constituents in small concentrations [52].
EO of other Myrtaceae plants containing α- and β-pinene, 1,8-cineole, linalool and α-terpineol (which were identified in the EO of our study) showed variable antimicrobial activities against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* [40]. EO of *P. racemosa* leaves from Benin exhibited antimicrobial activity against *E. coli* and *S. aureus* in spite of the difference in EO composition [6].
To sum up, the biological properties of the *P. racemosa* flower EO can be attributed to the complex mixtures of numerous molecules, compared to 1,8-cineole. This emphasizes the theory that natural products action is potentiated by synergism. In other words, the variation of concentrations and compositions within each EO is necessary for its action.

Table 3: Antimicrobial activity of *P. racemosa* flower essential oil

Microorganism	Inhibition zone diameter in mm ±SD (% of inhibition)			
	***P. racemosa* flower oil**	**Ampicillin**	**Gentamycin**	**Amphotericin B**
G +ve bacteria				
S. aureus	22.3 ±2.1 (81)	27.4 ± 0.18 (100)	N/A	N/A
B. subtilis	23.4 ±1.2 (72.2)	32.4 ± 0.10 (100)	N/A	N/A
G -ve bacteria				
P. aeruginosa	---	N/A	17.3 ± 0.15 (100)	N/A
E. coli	14.6 ±0.72 (65.4)	N/A	22.3 ± 0.18 (100)	N/A
Fungi				
A. fumigatus	19.2 ±1.2 (81.0)	N/A	N/A	23.7 ± 0.10 (100)
P. italicum	21.3 ±0.58 (97.2)	N/A	N/A	21.9 ± 0.12 (100)
G. candidum	22.4 ±1.5 (78.0)	N/A	N/A	28.7 ± 0.22 (100)

---: No activity and N/A is non-applicable

Table 4: MIC of *P. racemosa* flower essential oil.

Microorganism	MIC (µg/ml)	
	***P. racemosa* flower oil**	**Standard drug**
G +ve bacteria		**Ampicillin**
S. aureus	0.98	0.49
B. subtilis	0.49	0.49
G -ve bacteria		**Gentamycin**
P. aeruginosa	---	15.56
E. coli	6.22	0.98
Fungi		**Amphotericin** B
A. fumigatus	3.9	0.98
P. italicum	1.95	0.98
G. candidum	0.98	0.98

---: No activity

4. Conclusion
Fifty two components were identified by GLC-MS analysis of *P. racemosa* flower oil where 1,8-cineole was the major components. The oil showed potential antioxidant activity compared with ascorbic acid. It also showed diagnostic activity against different human carcinoma cell lines and antimicrobial activities against bacteria and fungi. *P. racemosa* flower oil could be a useful source of natural antioxidants and antimicrobial. Further studies are required to investigate the possible use of this oil as anticancer agent.

5. Conflict of interest
The authors declare no conflict of interest.

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