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Profiling of Volatile Constituents and Biological Activity Studies of *Ruprechita polystachya* Griseb Cultivated in Egypt

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Abstract

This study aimed to assess the volatile components and bioactivity potential of *Ruprechita polystachya* Griseb cultivated in Egypt. Air dried leaves was extracted was dichloromethane:methanol 1:1. Gas chromatography mass spectrometry (GC-MS) was applied to study the chemical constituents of n-hexane fraction while standard *in vitro* bioassays were used to detect the cytotoxic, antioxidant and antimicrobial potential of *R. Polystachy.* GC-MS analysis of n-hexane fraction showed the tentative presence of twenty-eight secondary metabolites classified as follow: ten fatty acid esters, eight hydrocarbones, two diterpenes, one triterpene, one phenyl propanoid, one phenolic derivative, one alkyl phenyl ketone, one α–tocopheroid, one γ-lactone derivative, one ketonic compound and one steroidal compound. The major identified compounds were: Ethenone-1-(2,3-dihydroxy-4-methoxy-6-methyl phenyl) (xanthoxylin) (49.41%), eugenol (4.89%), 9-octadecenoic acid (z)-methyl ester (3.18), hexadecenoic acid methyl ester (2.64%) and 9,12- octadecadienoic acid(z,z)-methyl ester (2.44%). *R. polystachya* extract showed cytotoxic activity against HeLa, MCF-7, HepG-2, PC3 and HCT-116 cancer cell lines with IC_{50} 10.20, 12.79, 16.10, 26.38 and 28.23 µg/ml resprctively. In antioxidant assay using ascorbic acid as areference drug, *R. polystachya* extract exhibited a high potentiality with IC_{50} 34.59 µg/ml while the corresponding fractions showed less potentiality. The antimicrobial activity of the extract/fractions was assessed using agar diffusion assay and crude extract showed a good inhibition against *C. Albicans* with activity index 74.1 % while it showed less activity against bacterial strains, *E. Coli* and *B. Subtilis*, with activity indicies 34.6 and 60.9 % respectively. *R. polystachya* can be considered as a potential source of bioactive compounds and more comprehensive exploration of its bioactive secondary metabolites is recommended.

Keywords: *Ruprechita polystachya*; volatile constituents; cytotoxicity; antioxidant; antimicrobial; *in vitro* activity.

1. Introduction

Natural products (NPs) are largely spread worldwide in natural sources as microorganisms, marine organisms, and plants [1]. Over the past decades, NPs have played significant parts in the development of numerous treatments of human diseases and health disorders. NPs are believed to be a major origin for bio-prospecting and introduction of new drug leads [2-6]. Historically, plants have been the key sources of several pharmaceutical agents, and cosmetic products. During the period from 1981 to 2014, FDA has approved many drugs derived from different medicinal plant species [7], including paclitaxel (Taxol®), docetaxel, topotecan, irinotecan, vinblastine(VBL), vincristine (VCR), vinorelbine (VRL), and etoposide [8, 9].

Ruprechtia is a genus belonging to Polygonaceae family and reported to possess several biological activities as cytotoxic, anti-inflammatory, antimicrobial, antioxidant and antiviral activities. Those biological activities were attributed to their bioactive constituents as tannins, terpenoids and flavonoids [10-12]. *Ruprechita* genus is one of the smallest and least explored Polygonaceae genera and comprises 17 species. *Ruprechtia polystachya* Griseb. (Polygonaceae) is a tree distributed from Mexico to northern Argentina and Uruguay where it is known as

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"vivaro bianco", "vivaro crespo", or "marmelero"; it is also widespread especially in Spain and Egypt, as ornamental tree [13].

This study was planned to investigate *R. polystachya* cultivated in Egypt for possible cytotoxic, antioxidant and antimicrobial activity as well as profiling of its volatile constituents using GC-MS which offers systematic evidence for commercial uses of the plant. Also studying for individual compounds may offer important drug leads but this step still under investigation, and will be reported in a fellow research work after further scrutiny.

2. Experimental

2.1. Chemicals

All the chemicals used in the extraction and fractionation procedures were of analytical grade, obtained from Sigma-Aldrich (St. Louis, MO, USA), and used as received. HPLC-grade solvent was used for the GC-MS analysis.

2.2. Plant material

The plant material was collected in March 2019 from Orman Botanic Garden, Giza region, Egypt, and identified by Ms. Therese Labib, Consultant at Orman Botanical Garden and El Qubba Botanical Garden, Giza region, Egypt. A voucher specimen (no. RP2019) of the plant has been deposited at the Herbarium of the Orman Botanical Garden, Giza region, Egypt.

2.3. Processing of the plant material

Two-week dried leaves was ground to fine powder (100 g) and extracted at room temperature with dichloromethane:methanol (1:1) for three days (whole process of extraction was done thrice). It was filtered out, and the filtrate was concentrated in vacuo by rotary evaporator to obtain a dark green crude extract (12 g). The crude extract was suspended in water (1 L) and fractionated using n-hexane (3.1 g), chloroform (4.3 g) and butanol (3.5 g). The obtained extracts were abbreviated as RP-LE (R. polystachya leaves extract), RP-H (R. polystachya hexane extract), RP-C (R. polystachya chloroform extract), and CP-B (R. polystachya butanol extract).

2.4. Gas chromatography-mass spectrometry analysis

The chemical composition of RP-H was analyzed by Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). Column oven temperature of the was initially set at 50°C and then increased gradually by 5°C /min to 250 °C and hold for 2 min. then increased to the last temperature 300°C by 30°C /min hold for 2 min. MS transfer line and injector temperatures were retained at 260 and 270 °C respectively; Helium gas was employed as a carrier with a stable flow rate of 1 ml/min. Delay of solvent was 4 min. and automatic injection of diluted samples of 1 µl was performed using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were obtained at 70 *eV* ionization voltages within the range of *m/z* 50–650 in the full scan mode. Temperature of the ion source was kept at 200 °C. Identification of the components was achieved by comparison of the retention times (RT) and mass spectra (MS) with those reported WILEY 09 and NIST 14 mass spectral database [14].

2.5. Cytotoxicity assay

2.5.1. Cell lines

Five cancer cells namely; Hepatocellular carcinoma (HePG-2), Epitheliod Carcinoma (Hela), Mammary gland (MCF-7), Human prostate cancer (PC3) and Colorectal carcinoma (HCT-116). The cell lines were purchased from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

2.5.2. MTT assay

The above mentioned cells were used to define the inhibitory effects on cell growths using MTT assay. It is a colorimetric assay built on the change of the yellow color tetrazolium bromide (MTT) to a purple color of formazan derivative *via* mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultivated in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added in concentrations as follow: 100µg/ml streptomycin and 100 units/ml penicillin at 37 ^{o}C in a 5% CO_2 incubator. The seeding procedure of cell lines was performed in 96-wells at a density of $1.0x10^4$ cells/well at 37 ^{o}C for 48 h under 5% CO_2. After incubation, different concentration of crude extract/fractions were added to the cells and incubated for 24 h. After 24 h of treatment, 20 µl of solution of MTT at 5mg/ml was loaded and incubated

for additional 4 h. 100 µl of dimethyl sulfoxide (DMSO was added to each well separately to dissolve the formed purple formazan. This assay is measured and noted at 570 nm absorbance by a plate reader (EXL 800, USA). Relative cell viability percentage was calculated as follow (A570 of treated samples/A570 of untreated sample) X 100 [15, 16]. Doxorubicin was used as a reference drug. The reagents MTT, RPMI-1640 medium and DMSO are purchased from sigma company (St. Louis, USA) while Fetal Bovine serum is from (GIBCO, UK).

2.6. DPPH free radical scavenging activity

The compounds' ability to donate hydrogen atom or electron was evaluated by the decolorizing of the purple solution of diphenylpicrylhydrazyl (DPPH). It is a spectrophotometric analysis using the stable radical of DPPH as a reagent. The samples were dissolved in methanol in a concentration of 1 mg/mL. To 0.4 mL of 0.1 mM methanolic DPPH, 200 mL of each sample were added and incubated for 30 min in the dark then the absorbance was recorded *versus* a blank at 517 nm [17]. Ascorbic acid was employed as a standard anti-oxidant. Blank sample, without DPPH, was run using only methanol. Negative control was run with methanol without the tested extracts. Calculation of the radical scavenging activity was achieved based on the following equation:

% Inhibition = ($A_{blank} - A_{sample}$)/(A_{blank}) x 100

2.7. Antimicrobial activities

The anti-bacterial activity of the extract/fractions was tried against [*Bacillus subtilis* (*B. subtilis*)] and [*Escherichia coli* (*E. coli*)] while the anti-fungal activity was tested against [*Candida albicans* (C. *albicans*)] by the agar diffusion technique [18, 19]. Crude extract as well as fractions were dissolved in DMSO and solutions of concentration 1 mg/ml were separately prepared on autoclaved paper discs of Whatman filter paper in a standard size (5 mm). The paper discs were soaked in the desired concentration and placed aseptically in the petri dishes having nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *B. subtilis* , *E. coli* and *C. albicans.* The petri dishes were incubated at 36 ºC and the inhibition zones were read after 24 h of incubation. Each experiment was done in triplicates. Antibacterial and antifungal activity of ciprofloxacin and colitrimazole were recorded under the same procedure, concentration and solvents as a standard reference drugs. The % activity index for the complex was calculated by the formula as under:

%Activity Index = Zone of inhibition by test compound (diameter)/ Zone of inhibition by standard (diameter) x 100

3. Results and discussion

3.1. GC/MS analyses of the n-hexane of R. polystachya

The chemical constituents of *n*-hexane fraction of *R. polystachya* were characterized by the comparison of their mass spectra with related counterparts reported by NIST, Wiley9, Mainlib, Replib libraries and/or authentic spectra [14]. Analysis of the n-hexane fraction of *R. polystachya* by GC-MS revealed the occurrence of twenty-eight compounds (Table 1, Figures 1 & 2), resembling 81.43% of the total fraction composition. Ethenone-1-(2,3-dihydroxy-4-methoxy-6-methyl phenyl) (xanthoxylin) (49.41%), eugenol (4.89%), 9-octadecenoic acid (z)-methyl ester (3.18), hexadecenoic acid methyl ester (2.64%) and 9,12- octadecadienoic acid(z,z)-methyl ester (2.44%) were the main components in *n*-hexane fraction.

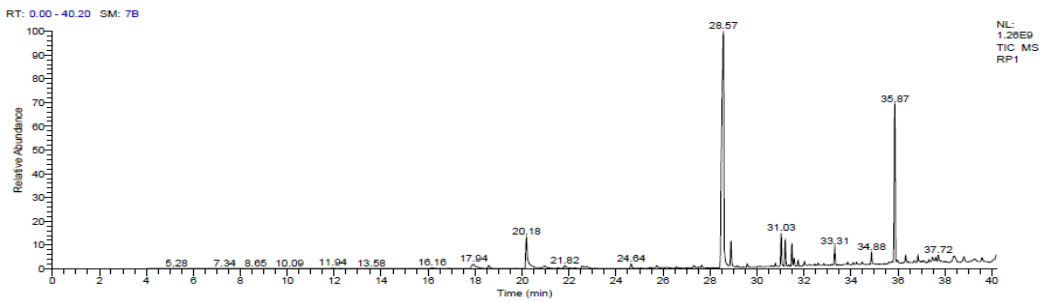

Figure 1: GC-MS chromatogram of the n-hexane fraction.

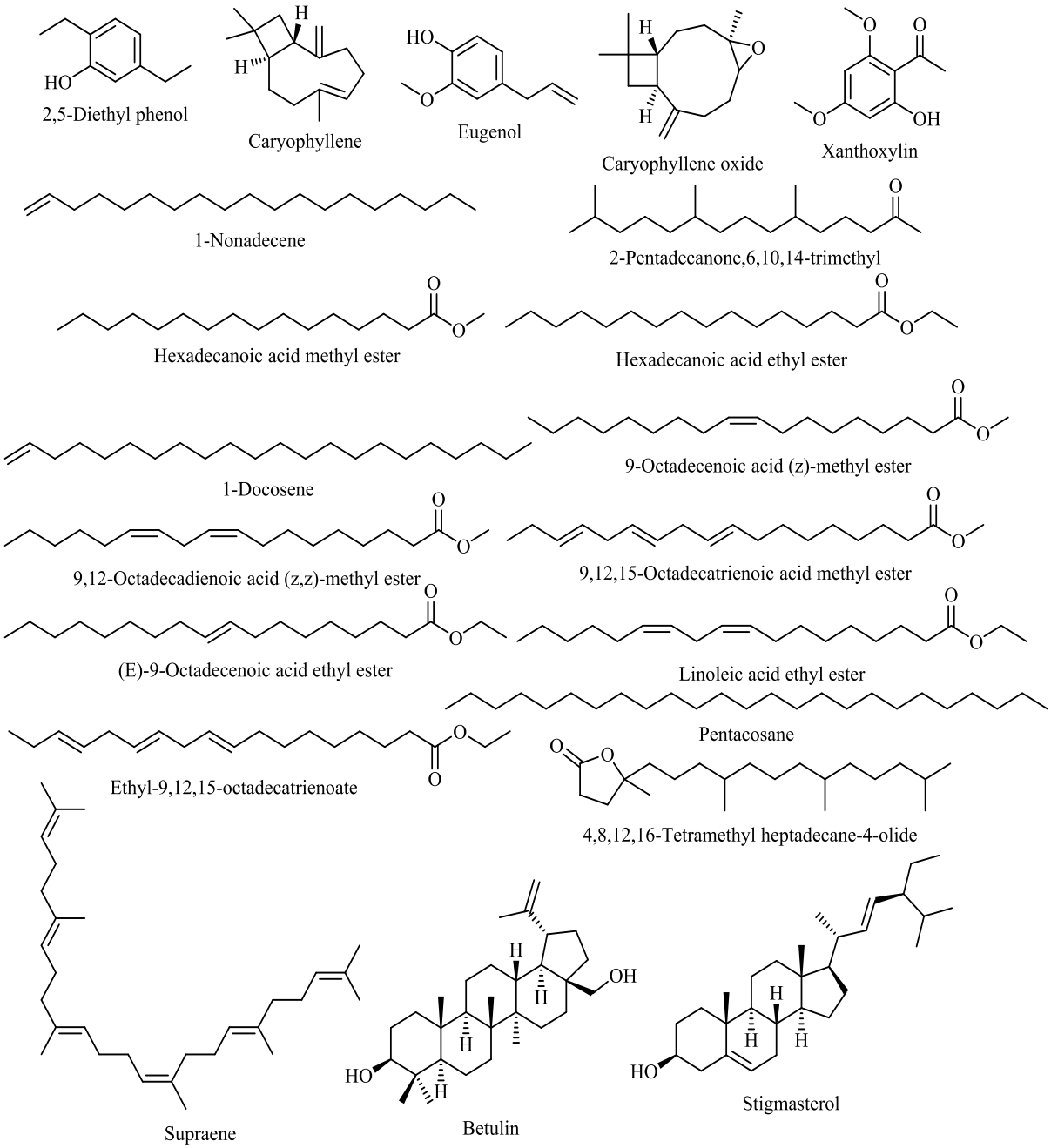

Figure 2: chemical structures of compounds depicted from the GC-MS.

3.2. Cell viability test

In this study, the cytotoxic effect of *R. polystachya* leave extract (RP-LE) and its subsequent fractions (RP-H, RP-C and RP-B) were tested by MTT assay on HepG-2, MCF-7, HCT-116, PC3 and HeLa cancer cells [20]. The results presented in Table 2 showed that leave extract had the highest potency to inhibit the viability of all tested cell lines in comparison to fractions. Noticeably, HeLa cell line was the most sensitive for Leave extract as well as butanol, n-hexane and chloroform fractions with an IC_{50} value of 10.20, 48.41, 56.59, 72.43 µg/ml respectively. On the other hand, HCT-116 and PC3 cell lines were the most resistant to both crude leave extract and its fractions (Table 2) while HepG-2 and MCF-7 cancer cells were moderately affected ones.

Table 1: MS data of compounds identified by GC/MS analyses of n-hexane fraction of *R. polystachya:*

No.	RT (min)	Proposed Compound	Area%	Mol. formula	Mol. mass
1	17.93	2,5-Diethyl phenol	1.33	$C_{10}H_{14}O$	150
2	18.59	Caryophyllene	0.48	$C_{15}H_{24}$	204
3	20.18	Eugenol	4.89	$C_{10}H_{12}O_2$	164
4	24.64	Caryophyllene oxide	0.65	$C_{15}H_{24}O$	220
5	25.73	1-Nonadecene	0.42	$C_{19}H_{38}$	266
6	27.65	2-Pentadecanone,6,10,14-trimethyl	0.32	$C_{18}H_{36}O$	268
7	28.56	Xanthoxylin	49.41	$C_{10}H_{12}O_4$	196
8	28.89	Hexadecanoic acid methyl ester	2.64	$C_{17}H_{34}O_2$	270
9	29.59	Hexadecanoic acid ethyl ester	0.42	$C_{18}H_{36}O_2$	284
10	30.78	1-Docosene	0.30	$C_{22}H_{44}$	308
11	31.03	9-Octadecenoic acid (z)-methyl ester	3.18	$C_{19}H_{36}O_2$	296
12	31.21	9,12-Octadecadienoic acid (z,z)-methyl ester	2.44	$C_{19}H_{34}O_2$	294
13	31.49	9,12,15-Octadecatrienoic acid methyl ester	1.98	$C_{19}H_{32}O_2$	292
14	31.58	(E)-9-Octadecenoic acid ethyl ester	0.82	$C_{20}H_{38}O_2$	310
15	31.75	Linoleic acid ethyl ester	0.54	$C_{20}H_{36}O_2$	308
16	32.03	Ethyl-9,12,15-octadecatrienoate	0.68	$C_{20}H_{34}O_2$	306
17	33.31	Pentacosane	1.85	$C_{25}H_{52}$	352
18	33.86	4,8,12,16-Tetramethyl heptadecane-4-olide	0.30	$C_{21}H_{40}O_2$	324
19	34.88	Docosane	1.33	$C_{22}H_{46}$	310
20	35.99	Tetracosanoic acid methyl ester	0.27	$C_{25}H_{50}O_2$	382
21	36.33	Heptacosane	0.99	$C_{27}H_{56}$	380
22	36.86	2,6,10,14,18,22-Hexamethyl oxirane (Supraene)	0.91	$C_{30}H_{50}$	410
23	37.32	Oxiraneoctanoic acid-3-octyl cis	0.25	$C_{18}H_{34}O_3$	298
24	37.48	α-Tocopiro B	0.73	$C_{29}H_{50}O_4$	462
25	37.72	Dotriacontane	0.66	$C_{32}H_{66}$	450
26	38.40	Betulin	1.74	$C_{30}H_{50}O_2$	442
27	38.82	17-Pentatriacontene	1.06	$C_{35}H_{70}$	490
28	39.27	Stigmasterol	0.84	$C_{29}H_{48}O$	412
Total %			81.43%		

3.3. DPPH free radical scavenging assay

The DPPH is an assay used to evaluate the antioxidant potential of different chemicals. It is considered as an effective method to estimate the radical-scavenging action of a compound by a chain-breaking mechanism [21, 22]. This method depends on the ability of an antioxidant to decolorize DPPH. The results of the DPPH free radical scavenging activity listed in Table 3. The leave extract was the most active in this assay (IC_{50} 34.59 µg/ml) with comparable activity to that of ascorbic acid (IC_{50} 29.70 µg/ml). The three fractions showed lower radical scavenger activity with IC_{50} 70.23, 74.01 and 85.56 µg/ml for hexane, butanol and chloroform respectively.

Table 2: Influence of the extract/fractions of *R. polystachya* on the viability of different cancer cell lines:

Extract/Fractions	In vitro Cytotoxicity IC_{50} (µg/ml)•				
	HepG-2	MCF-7	HCT-116	PC3	HeLa
RP-LE	16.10±1.3	12.79±1.1	28.23±2.1	26.38±1.9	10.20±1.0
RP-H	76.25±4.3	45.40±2.9	67.54±3.8	63.26±3.9	56.59±3.8
RP-C	82.02±4.8	68.15±4.0	78.61±4.4	92.51±5.1	72.43±4.2
RP-B	50.23±3.3	53.43±3.5	65.72±4.1	84.65±4.7	48.41±3.0
Doxorubicin	4.50±0.2	4.17±0.2	5.23±0.3	8.87±0.6	5.57±0.4

• **IC_{50} (µg/ml)**: 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)

Table 3: *In vitro* DPPH free radical scavenging assay of the extract/fractions of *R. polystachya*:

Extract/Fractions	Conc. (µg/ml)						
	10	20	30	40	50	60	IC_{50}
RP-LE	16.8	29.3	40.3	51.5	65.4	77.4	34.59±0.19
RP-H	12.0	22.4	28.3	32.0	41.3	48.1	70.23±0.29
RP-C	19.5	25.0	31.6	33.1	35.2	50.4	85.56±0.32
RP-B	19.1	24.8	29.5	34.6	38.1	52.2	74.01±0.31
Ascorbic acid	19.7	33.9	48.1	57.6	69.3	81.5	29.70±0.13

3.4. Antimicrobial activity evaluation

The antimicrobial potential of the crude leave extract/fractions was evaluated against gram-negative bacteria *E. coli* and gram-positive bacteria *B. subtilis* and also against the pathogenic fungus *C. albicans*. A standard agar diffusion technique was employed [23] and the diameters [mm] of inhibition zones are summarized in Table 4. Obviously, the leave extract was the most active and showed the highest activity indices 34.6, 60.9 and 74.1% against *E. coli*, *B. subtilis* and *C. albicans* respectively in comparison to ciprofloxacin (antibacterial) and clotrimazole (antifungal) as a reference drugs. Butanol fraction RP-B came in the second place after the leave extract with 7.7, 21.7 and 18.5% activity index against *E. coli*, *B. subtilis* and *C. albicans* respectively. The third place was occupied by hexane fraction while chloroform fraction was the least active fraction. It is also clear that the fungal strain *C. albicans* was the most sensitive and showed the highest inhibition by *R. polystachya* leaves extract/fractions while *B. subtilis* was moderately inhibited and the highest resistance was shown by the pathogenic gram-negative bacteria *E. coli*.

Table 4: Antimicrobial activities of the extract/fractions of *R. polystachya* using agar diffusion assay:

Extract/Fractions	Diameter inhibition zone in mm (% activity index)		
	E. coli	***B. subtilis***	***C. albicans***
RP-LE	9 (34.6)	14 (60.9)	20 (74.1)
RP-H	NA (----)	3 (13.0)	7 (25.9)
RP-C	NA (----)	NA (----)	4 (14.8)
RP-B	2 (7.7)	5 (21.7)	5 (18.5)
Ciprofloxacin	26 (100)	23 (100)	----
Clotrimazole	----	----	27 100)

4. Conclusion

R. polystachya was investigated for its volatile secondary metabolites composition using GC-MS which indicated the presence of twenty-eight secondary metabolites and the major identified compounds were: Ethenone-1-(2,3-dihydroxy-4-methoxy-6-methyl phenyl) (xanthoxylin) (49.41%), eugenol (4.89%), 9-octadecenoic acid (z)-methyl ester (3.18), hexadecenoic acid methyl ester (2.64%) and 9,12- octadecadienoic acid(z,z)-methyl ester (2.44%). *R. polystachya* also was evaluated for cytotoxic activity against HeLa, MCF-7, HepG-2, PC3 and HCT-116 cancer cell lines with IC_{50} 10.20, 12.79, 16.10, 26.38 and 28.23 µg/ml respectively. antioxiant and antimicrobial activity. *R. polystachya* showed moderate to weak activity in all assays. These results assumed that *R. polystachya* can be considered as a source for bioactive compounds and calls for further investigation and isolation of chemical constituents which may afford potential drug leads against different diseases.

5. list of abbreviations

Abbreviations	Title
R. Polystachya	*Ruprechita polystachya*
GC-MS	Gas chromatography mass spectrometry
HePG-2	Hepatocellular carcinoma
Hela	Epitheliod Carcinoma
MCF-7	Mammary gland
PC3	Human prostate cancer
HCT-116	Colorectal carcinoma
DPPH	2,2-diphenyl -1-picrylhydrazyl
RP-LE	*R. polystachya* leaves extract
RP-H	*R. polystachya* hexane extract
RP-C	*R. polystachya* chloroform extract
RP-B	*R. polystachya* butanol extract

6. Conflicts of interest

There are no conflicts to declare.

7. References

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