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Chemical Composition of Essential Oil and *in vitro* Antibacterial and Anticancer Activity of the Hydroalcolic Extract from *Coronilla varia*

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***Abstract*—**The aims of study were investigation on chemical composition essential oil and the effect of extract of *Coronilla varia* on antimicrobial and cytotoxicity activity. The essential oils of *Coronilla varia* is obtained by hydrodistillation and analyzed by (GC/MS) for determining their chemical composition and identification of their components. Antibacterial activity of plant extract was determined by disc diffusion method and anticancer activity measured by MTT assay. The major components in essential oil were Caryophyllene Oxide (60.19%), Alphacadinol (4.13%) and Homoadantaneca Robexylic Acid (3.31%). The extracts from *Coronilla varia* had interesting activity against *Proteus mirabilis* in the concentration of 700 µg/disc and did not show any activity against *Staphylococus aureus, Bacillus subtillis, Klebsiella pneumonia* and *Entrobacter cloacae*. The positive control, Ampicillin, Chloramphenicol and Cenphalothin had shown zone of inhibition resistant all bacteria. The ethanol extract of *Corohilla varia* inhibited on MCF7 cell lines. IC_{50} 0.6(mg/ml) was the optimum concentration of extract from *Coronilla varia* inhibition of cell line growth. The MCF7 cancer cell line and *Proteus mirabilis* were more sensitive to *Coronilla varia* ethanol extract.

Keywords*—Coronilla varia*, Essential oil, Antibacterial, Anticancer, HeLa cell line.

I. Introduction

THE plant-derived compounds have always been an important source of medicines for various diseases and have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects [1]. During the last few years, novel chemopreventive agents of natural origin have been targeted with fruits and vegetables being a key interest due to high content of bioactive compounds [2]. Cancer is the second leading cause of death all over the word [3]. According to World Health Organization, more than 10 million new cases of cancer are diagnosed every year ,and the statistical trends indicate that this number would double by 2020 [4]. Cancer is the uncontrolled growth and spread of abnormal cells, associated with dis regulation of apoptosis, a programmed cell death. Most of the current anticancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity [5]. Essential oils used in traditional therapies are also called volatile oils are generally aromatic oils obtained by the steam or hydrodistillation of plants. Different parts of plants have been used to obtain essential oils. Essential oils and their components are widely used in medicine as constituents of different medical products in the food industry as flavoring additives and also in cosmetics as fragrances and pharmaceutical industries [6], [5]. These include flowers, leaves, seeds, roots, stems, bark and wood though secretionary parts.

Antibiotic resistance has become a global concern. Moreover screening of such plant extracts for antimicrobial activity has always been of great interest to scientists looking for new sources of drugs for the treatment of various microbial diseases [7], [8].

Coronilla varia (Crown vetch) is in the legume family. It is perennial and it forms thick vegetation. It has pink and white small flowers in early summer. This plant was introduced into the United States for erosion control. It is now considered an invasive plant. There are several medicinal claims for crown vetch such as folk medicine treatment of prostrate disorders and as a diuretic heart tonic. Crown vetch is toxic to horses because of the presence of nitroglycosides. If consumed in large amounts, it can cause slow growth, paralysis, or even death. The whole plant contains a toxic glycoside called coronillin. It is one of the most toxic plants growing in Britain [9]. The present study was aimed at investigation on chemical composition essential oil and the effect of aqueous-ethanolic extract of *Coronilla varia* on antimicrobial activity and against MCF7 cancer cell line.

II. Materials and Methods

The flower of *Coronilla varia* were collected from Gadook Firoozkooh (North of iran), Iran, in the summer of 2014. The samples were identified by Dr. Bahman Eslami (Assistant Prof. of plant systems, Islamic Azad University of Qaemshahr, Iran). Voucher specimens are deposited with the faculty of biology herbarium (vol.157p.p359).

A. Isolation of the Essential Oil

The flowers of the plant collected were submitted for 3 h to water-distillation using a British-type Clevenger apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and after filtration, stored at 4°C until tested and

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analyzed.

B. Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS was carried out using a Hewlett- Packard 5975B series instrument and an Agilent 19091J-433 HP-5 capillary column (30 m., 250 m i.d., film thickness 0.25m) which was set at 50°C for 10 min, then increased 4°C/min to 300°C; using Helium as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was m/z 40–400. The compounds were identified according to their retention indexes and by comparison of their mass spectra with those of a computer library or with authentic compound.

C. Preparation of Ethanolic Extract

The flowers were dried at room temperature before extraction. A known amount of flowers was extracted at room temperature following the percolation method using ethanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained (10.8 %), which was then freeze-dried for complete solvent removal.

D. Assay for Antibacterial Activity of Plant Extract

Antibacterial assay of ethanol extract is measured by disc diffusion method as described by Bauer [10]. Three Gram negative bacteria (*Proteus mirabilis* PTCC (1076); *Enterobacter cloacae* PTCC (1003), and *Klebsiella pneumonia* PTCC (1290)) and two Gram positive bacteria (*Staphylococcus aureus* PTCC (1112) and *Bacillus subtilis* PTCC (1023)) were used for the present study. All the test bacteria were collected from Pastor Institute of Iran. Dried filter paper discs (4mm in diameter) impregnated in known amount of test substances (400 µg/discs) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Ampicillin, Cephalothin and Cholrampnicol discs (30µg/disc) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4ºC) for two to four hours to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel the plates were then incubated at 37ºC for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called “Zone of Inhibition”. The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

E. Cytotoxicity Assay

An MTT assay was employed to evaluate the cytotoxic potential of the extracts. MCF7carcinoma cell lines were used for the MTT assay. Cells (1 × 105 cells/well) were incubated for 72 h with the test sample at least 5 extract concentration. The MTT solution (0.5 mg/mL) was added, followed by further incubation for 4 h. The medium was then removed and the wells were washed with PBS. DMSO (200 /L) was added to each well and the absorbance of each well was measured at 570 nm using a micro plate Elisa reader. The percentage of cytotoxicity is calculated as [(A-B)/A]x100, where A and B are the OD570 of untreated and of treated cells, respectively.

F. Data analysis

IC_{50} of cytotoxicity assay were obtained from dose-effect-curves (not shown). The IC_{50} are the average of four assays with 5 concentrations within the inhibitory range of the compound. The therapeutic index (i.e. selective index) is defined as IC_{50}.

III. Results and Discussion

The results obtained by the GC-MS analysis of the essential oil of the *Coronilla varia* aerial parts are presented in Table I. Twenty three compounds were identified, representing 98.8% of the total oil. The oil yield of the plant was determined as 0.94% v/w. As determined from the GC-MS analysis. The major components were Caryophyllene Oxide (60.19%), Alphacadinol (4.13%) and Homoadantaneca Robexylic Acid (3.31%).

TABLE I
THE MAJOR COMPONENT IN THE ESSENTIAL OIL OF *CORONILLA VARIA*

No	components	R.T	KI	%
1	Z-Citral	18.534	1449	1.800
2	Nerol	22.344	1589	1.86
3	Trans-beta-Farnesene	23.244	1624	1.02
4	3-Butenamide	25.158	1701	1.13
5	Caryophyllene oxide	26.62	1760	2.11
6	Caryophyllene oxide	27.248	1790	8.62
7	Caryophyllene oxide	27.299	1792	44.08
8	Aromadendrene Veridiflorol	27.513	1801	2.41
9	3-Cyclohexen-1-caroxaldehyde	27.907	1819	1.96
10	cis-3a,4,5,6,7,7a-Tetrahydro-1H-inden-1-one	28.548	1847	1.21
11	Tau-muurolol	28.645	1852	1.75
12	alpha-Cadinol	28.942	1865	4.13
13	1-Homoadamantaneca	29.027	1869	2.13
14	Tricyclo[4.3.1.13,8]undecane-1-car boxylic acid	29.331	1869	2.13
15	6-butyl-3,6-dihydro-2-(1h)-pyridinone	29.589	1882	3.31
16	Palustrol	30.514	1894	1.83
17	2-dimethylamino-3-methyl-4-(3h)pyrimidinone	30.812	1937	1.94
18	Benzenemethanol	31.245	1971	1.44
19	(E,Z)-ALPHA-FARNESENE	31.368	1977	4.04
20	Caryophyllene oxide	31.478	1982	5.38
21	2-Pentadecanone	32.850	2050	2.22
22	1,2-Benzenedicarboxylic acid	33.406	2077	1.91
23	Di-(2—etylhexyl)phthalate	45.172	2756	1.95

A. Assay for Antibacterial Activity

The *in vitro* antimicrobial activity of crude extract of *Coronilla varia* on Gram-positive and Gram-negative bacteria, collected from Firozkoh, was studied. Crude extract of *Coronilla varia* (700 mg/disc) showed moderate activity against only one bacterium. These results are shown in Table II. The maximum activity was on *Proteus mirabilis* PTCC(1076) (10 mm) and no activity was found on *Enterobacter cloacae* PTCC(1003), *Klebsiella pneumonia* PTCC(1290), *Staphylococcus aureus* PTCC (1112) and

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Bacillus subtillis PTCC(1023).The positive control showed in Table II.

TABLE II
ANTIBACTERIAL ACTIVITY OF *CORONILLA VARIA* ETHANOL EXTRACT AS MINIMUM INHIBITORY CONCENTRATIONS (MICS) in- G/ML

	Coronilla varia ethanol extract (700 µg /disc) (mm dimater)	Cephalothin (15µl/ disc) (mm dimater)	Ampicilin (15 µl /disc) (mm dimeter)	Choramphenicol (15µl /disc) (mm dimater)
Proteus mirabilis PTCC(1076)	10	32	0	17
Enterobacter cloacae PTCC(1003)	0	9	0	29
Klebsiella pneumonia PTCC(1290)	0	12	0	29
Staphylococcus aureus PTCC (1112)	0	30	0	33
Bacillus subtillis PTCC(1023)	0	35	0	35

B. Assay for Anticancer Activity

The effect of ethanol extracts from *Coronilla varia* was investigated on MCF7 cancer cell line. The cytotoxicity assay of ethanol extract from *Coronilla varia* was measured by MTT assay. Ethanolic extract of *Coronilla varia* L. has significant cytotoxicity effect on MCF7 cell line (Table III). IC50 value of *Coronilla varia* L. on MCF 7 cell was 0.61 mg/ml by MTT assay.

TABLE III
THE RESULTS OF CYTOTOXICITY ETHANOL EXTRACTS OF *CORONILLA VARIA* INVESTIGATED ON MCF7 CANCER CELL LINE IN DIFFERENT CONCENTRATION

Concentrations of *Coronila varia* (mg/ml)	Absorbance	% inhibition	IC_{50}
0. 125	0.543±0.041	10.14	
0.25	0.02±0.595	17.45	
0.5	0.573±0.039	15.15	
1	0.027±0.60	40.9	0.6(mg/ml)
2.5	0.64± 0.048	63.75	
5	0.68±0.015	78.95	
7.5	0.73±0.045	83.1	
10	0.76± 0.012	87.6	
Control	0.534±0.0279		

VI. DISCUSSION

Twenty three compounds that major components were identified in the essential oil, representing 98.8% of the total oil. Maybe local climate and / or the increase in temperature to 300^{o}C have played a major role in the number of identified components [11]. The antimicrobial activities of various plants have been reported by [12]. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism. In the present study, a variety of gram positive and gram negative strains were selected for screening antimicrobial effects of ethanolic extract of *Coronilla varia*. The result of this study showed that the ethanolic extract of exhibited varied range of antimicrobial activity against the tested organism including gram positive and gram negative bacteria, which is comparable to standard antibiotic effect. The *Coronilla varia* extracts exhibited the greatest antimicrobial activities (as determined by the diameters of the inhibition zones towards susceptible bacteria like Proteus mirabilis and did not show any activity in *Enterobacer cloacae, Klebsilla pneumonia* and *Staphylococeus aureus*. The therapeutic effect of herbal materials in inhibition of cancer cell growth was shown. This study investigates the effect of ethanolic extract from *Coronilla varia* on MCF7 cancer line. Cancer cell line was more sensitive to ethanolic extract from *Coronilla varia* .The *Coronilla varia* ethanol extract could inhibit the proliferation of MCF7 cell line in RPMI 1640 medium. 0.6(mg/ml) was the optimum concentration in inhibition of cell line growth. The MCF7 cancer cell line was more sensitive to *Coronilla varia* methanol extract. This anticancer activity might be due to presence of its proteolytic enzymes [13].

V. CONCLUSION

In summary, pharmacological evaluation of *Coronilla varia* extract reveals some interesting activities like cytotoxicity and antibacterial activities of this plant. Since, crude ethanol extract of *Coronilla varia* showed cytotoxicity and antibacterial effect, we assume that different active secondary metabolites are present in its extracts may function in a synergistic manner. Further studies should be going on fractionation and identification of bioactive constituent to human cell line culture of cytotoxic effect. This report may serve as a footstep regarding the biological and pharmacological activities of *Coronilla varia* .

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