SYNERGISTIC ACTIVITIES OF THE ESSENTIAL OILS HYPERICUM PERFORATUM WITH METHOTREXATE ON HUMAN BREAST CANCER CELL LINE MCF-7

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Hypericum perforatum (Hypericaceae) is a perennial plant usually known as "Sarikantaron" that has been reported to have various important biological activities.In this present study, it was aimed to (I) identify the components (ii) show the synergistic activities of the essential oils *Hypericum perforatum* with methotrexate on human breast cancer cell line MCF-7. A normal [Madin-Darby Bovine Kidney (MDBK) cell line] and a cancer cell line [Human breast adenocarcinoma cell line (MCF-7)] were used in this study. The cell culture were treated with various concentrations of *Hypericum perforatum*'s oils. The cytotoxic activity of the essential oils of *Hypericum perforatum* on the cell lines was measured using the MTT method and the results were evaluated as IC_{50}. In this study, the presence of trans-caryophyllene, germacrene-D, α-pinene, trans-cadina-1,4-diene, β-Selinene, caryophyllene oxide and α-Selinene were identified as major constituents of *Hypericum perforatum*'s oils. The essential oils of *Hypericum perforatum* also exhibited anticancer activities against MCF-7 cells. The IC_{50} values of the essential oils, MTX and the essentialoilsplus MTX weredetermined as 0.78, 6.25 and 0.195 µg/ml, respectively, But, the essential oils of *Hypericum perforatum* was found to be non-cytotoxic for MDBK cells. In conclusion, the essential oils of *Hypericum perforatum* was chemically characterized and γ-muurolene, δ-cadinene, germacrene B, α-copaene, germacrene D, bicyclogermacrene, and (E)-caryophyllene were found to be major constituents. The essential oils of *Hypericum perforatum* possess significant *in vitro* anticancer potential. The essential oils of *Hypericum perforatum* with MTX found to be significant effective against breast cancer cells. Further studies especially *in vivo* anticancer properties of *Hypericum perforatum* should be searched. These compounds found to be very promising compounds in the treatment of cancer therapy.

Keywords: MCF-7 cell line, anticancer, *Hypericum perforatum*, essential oil, synergistic activity.

INTRODUCTION

Breast cancer is one of the most common types of cancer in women. Also, it can be seen in both women and men. Breast cancer is the major cause of death among women (Siegel *et al*., 2014).

Cancer is one of the most important diseases of this of this century.Chemotherapy for the treatment of breast cancer is one of the most effective treatment options. Unfortunately, there is no effective drug for the treatment of certain cancers. There are common side effects of current drugs used in cancer treatment, as well as low efficiency in treatment. furthermore, drug resistance in cancer treatment is another serious problem (Raguz, 2008).

Therefore, the new drug researches for cancer chemotherapy continue. An important part of the studies on this topic has focused on natural products (Burmaoglu *et al*.,

2016). Because its many pharmacological features, *Hypericum perforatum* L. is interesting plant species for cancer research. *Hypericum perforatum* L. is a member of the *Hypericaceae* is reported to identify more than 400 species in the world (Mabberley, 1987). It can grow in many countries the world as well as in our country. *H. perforatum* is reported to have very important pharmacological properties among medicinal plants (Wills and Bone, 2000). Hypericum species reported to use in the folk medicine for the treatment of many diseases such as skin wounds, burns, eczema, gastrointestinal disorders and psychological disorders (Butterweck, 2003).

In this study, we aimed to investigate the anticancerogen activity of *Hypericum perforatum* on human breast adenocarcinoma (MCF-7) cell line. Also, it was aimed to search the synergistic activities of the essential oils *Hypericum perforatum* with MTX (Methotrexate) on MCF-7cells.

MATERIALS AND METHODS

Isolation Of The Essential Oils

Herba of *Hypericum perforatum* were collected from Amanos Mountain (Anatolian region) in blooming period and dried at room temperature. Essential oils were obtained from dried leaves and flowers. The aerial parts of *Hypericum perforatum* (100 g) were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and then stored in dark color glass bottles, at -4 ^{o}C ready for GC-MS analysis.

Analysis of GC/MS

Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, autosampler and TR-5MS (5% Phenyl Polysilphenylenesiloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 ^{o}C, MS Ionization source temperature was 220 ^{o}C, the injection port temperature was 220 ^{o}C. The samples were injected with 250 split ratio. The injection volume was 1 µL. Oven temperature was programmed in the range of 50 to 220 ^{o}C at 3 ^{o}C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley 9 library). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

Table 1. Essential oil components of *Hypericum perforatum*

RT	RI	Compound Name	Cas #	Area %
2,36	902	Nonane	111-84-2	1,28
2,93	970	Nonane, 3-methyl	5911-04-6	0,54
3,68	1031	α-Pinene	80-56-8	10,59
4,14	1060	Decane, 2-methyl	6975-98-0	0,81
4,94	1101	Undecane	1120-21-4	0,52
5,19	1114	β-Pinene	127-91-3	0,64
6,56	1172	β-Myrcene	123-35-3	0,66
9,15	1260	cis-Ocimene	6874-10-8	1,16
16,58	1465	α-Longipinene	5989-08-2	0,31
17,20	1480	α-Ylangene	14912-44-8	0,3
18,53	1514	β-Bourbonene	5208-59-3	0,26
20,41	1564	β-Sesquiphellandrene	20307-83-9	1,36
21,45	1590	trans-Caryophyllene	87-44-5	17,13
23,05	1634	β-Chamigrene	18431-82-8	0,46
23,19	1637	Valencene	4630-07-3	0,25
24,12	1662	α-Humulene	6753-98-6	1,05
24,38	1669	β-Farnesene	18794-84-8	2,69
25,00	1685	Junipene	475-20-7	0,21
25,59	1700	Germacrene-D	23986-74-5	11,22
25,91	1709	β-Selinene	17066-67-0	6,99
26,05	1714	trans-Cadina-1,4-diene	87-44-5	8,84
26,12	1716	α-Selinene	473-13-2	4,63
26,49	1726	Bicyclogermacrene	100762-46-7	1,36
26,59	1729	α-Bergamotene	17699-05-7	0,98
27,47	1754	β-Elemene	515-13-9	2,53
34,89	1969	Caryophyllene oxide	1139-30-6	5,26
37,26	2043	Nerolidol	7212-44-4	1,06
39,58	2126	Spathulenol	77171-55-2	0,77
39,84	2139	1-Dodecanol	112-53-8	3,93
39,99	2146	junipercamphor	NA	0,31
41,33	2206	1-Hexadecanol	36653-82-4	3,55
41,91	2221	Cubenol	21284-22-0	0,23
42,10	2226	α-Bisabolol	72691-24-8	0,39
42,42	2234	Longipinane	NA	0,56
42,84	2245	Veridiflorol	552-02-3	0,71
43,39	2259	Globulol	51371-47-2	1,6
47,01	2370	Junipene	475-20-7	0,38

Determination of Non-Toxic DMSO Concentration

To solve the plant essential oils, DMSO (dimethylsulfoxide) was used as a potent solvent. To determine the non-toxic concentration of DMSO on MDBK, 1×10^6 cells were inoculated into each well of flat bottomed plates. RPMI-1640 was selected as a growth medium. Plates were incubated 96 hours in the presence of decreasing amounts of DMSO (8%, 4%, 2%, 1%, 0.5%).

To determine the non-cytotoxic concentration of *Hypericum perforatum* Madin-Darby Bovine Kidney (MDBK) cell line was selected. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v). Cells were incubated in a humidified atmosphere at 37 ºC in 5% CO_2.

Cytotoxic Tests

For this purpose, the MTT assay was selected. MTT assay was performed as described previously (Mosmann, 1983). The normal and cancer cells were cultured in RPMI-1640 medium with 10% (w/v) fetal bovine serum. Incubation of the cells was performed at 37°C with 95% air and 5% carbon dioxide. The essential oils of the plants were dissolved in DMSO at the concentration of lower than 1%. The culture cell is inoculated in 96-well plates overnight. After 96 hours of incubation with plants esssential oils, the cells were washed with PBS. And then, 100 µL of 0.5 mg/mL MTT were added to all wells and incubated at 37 °C. The plate was incubated for 30 minutes at the same conditions. MTT reduction ratio was determined by measuring the difference in absorbance at 570 and 650 nm using a microplate reader. All tests were performed in triplicate.

Statistical Analysis

All data were obtained in the experiments repeated three times. Statistical analyses were performed using Student t-test. The p value<0.05 was considered significant. All statistics in the present study were done using SPSS program.

RESULTS AND DISCUSSION

The chemical composition of the essential oils investigated in this study is shown in Table 1. *Hypericum perforatum* has been a rich constituents such as trans-Caryophyllene (17.13%), Germacrene-D (%11.22), α-Pinene (10.59%), trans-Cadina-1,4-diene (8.84%), β-Selinene (6.94%), Caryophylleneoxide (5.26%) and α-Selinene (4.63%), 1-Hexadecanol (3.93%), β-Farnesene (2.69%), β-Elemene (2.53%), Nonane (1.28%) and Nerolidol (1.06%).

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Today, there are a limited numbers and drug activity in cancer chemotherapy. Methotrexate is a commonly used drug for the treatment of many cancers types such as breast cancer (http://www.breastcancer.org). Due to the many side effects of MTX and the low treatment success, new active molecules investigation continues intensively. We have investigated the anticancer activity and the existence of a synergistic effect of *Hypericum perforatum*'s oils with MTX in this study.

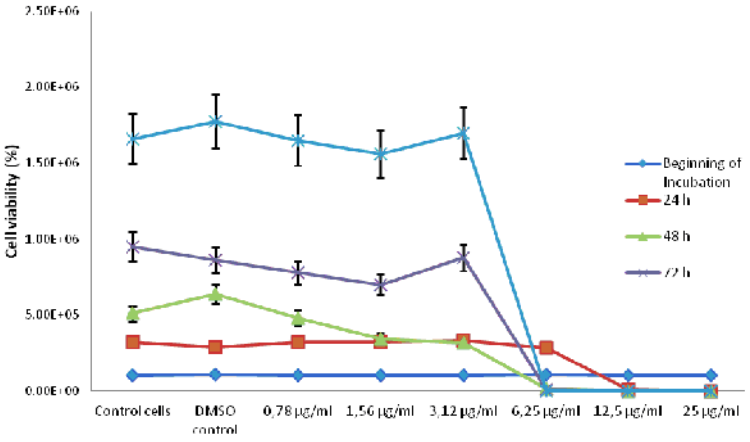

Figure 1. Effects of the essential oils of *Hypericum perforatum* on the proliferation of MCF-7 cells

The Inhibition concentrations of the essential oils of *Hypericum perforatum* against cancer (MCF-7) and normal cells (MDBK) cells were evaluated. The IC_{50} values were determined as 6.25 µg/ml and 25 µg/ml, respectively. The IC_{50} value for MTX against MCF-7 cells was determined to be 0.78 µg/ml. This value was calculated for the essential oils of *Hypericum perforatum* (EO of HP) plus MTX as 0.195 µg/ml. It had been found that the essential oils of *Hypericum perforatum* enhanced synergistically the effect of MTX against MCF-7 cells (Figure 2).

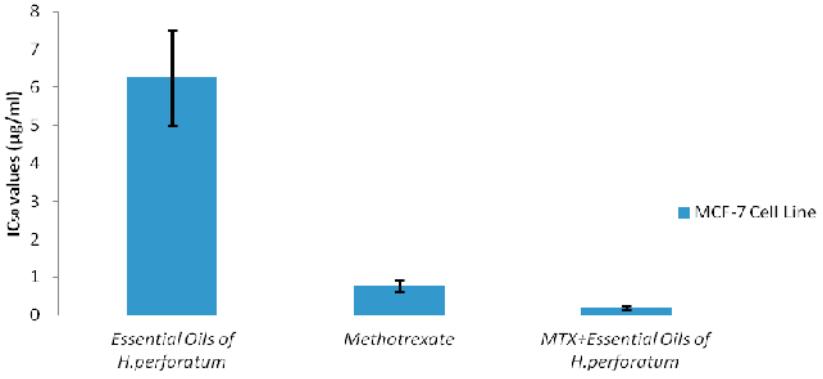

Figure 2. Effects of the essential oils of *Hypericum perforatum* on the proliferation of MCF-7 cells

In Figure 2, the synergistic effects of the methotrexate with the essential oils of *Hypericum perforatum* were investigated. As is clear in the figure 2, the essential oils of *Hypericum perforatum* significantly raised the effectiveness of the standard anticancerogen drug. A synergistic activity was determined between *the essential oils of Hypericum perforatum* and methotrexate.

CONCLUSIONS

In conclusion, *Hypericum perforatum* collected from the South of Turkey (Hatay region) quite inhibited human lung adenocarcinoma cells, and was not toxic to normal cells. Besides this, the strong synergistic activity between methotraxate and *Hypericum perforatum* was determined. We think that *Hypericum perforatum* L. may be a promising natural product for the breast cancer chemotherapy. However, further studies are needed on this issue.

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