Pharmacological Properties and Cytotoxic Effects of *Matricaria Chamomilla* Plant Extracts *by MTT* Assay

Duran Kala

Ishik University, Erbil, Iraq, Email: duran.kala@ishik.edu.iq

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Abstract: The paper describes pharamacolgical properties of therapeutic plants Matricaria chamomilla,is referred to as chamomile or German chamomile. It is used in folk medicine and in modern medicine as therapeutic plant in the thearapy of upper respiratory infections and various disorders. Chamomile can live in large areas in nature. Every herbal plant has cytotoxic effects. In determination of any possible cytotoxic effects of Matricaria chamomilla, extracts on HeLa CEACAM cells, serial solutions (1/10, 1/50, 1/100, 1/1000,) of Matricaria chamomilla, extracts were incubated with HeLa CEACAM cells for 24 and 48 h. The cell viability was calculated by the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Matricaria chamomilla, plant extracts showed toxic effect at 1/10 solution, however toxicity of the extracts definitely decreased at 1/50 solution for 24 and 48 hours. Results suggested that the cytotoxic effect of Matricaria chamomilla extracts were concentration dependent but not time-dependent in that less cells were viable at 1/10 solution compared to 1/1000 solution. According to results, the plant extract solution of 1/1000 was calculated as an effective dose for future researches.

Key Words: Chamomile, Matricaria Chamomilla, HeLa CEACAM, Cytotoxcity, MTT, Cancer

Introduction

Matricaria chamomilla, generally known as the chamomile or German chamomile, is a species of the Asteraceae family that consist of most species of flowering plants in scientific classification, and is one of the significant medicinal plant native to Europe and Western Asia (Renuka, 1992). The plants live in North Africa, Asia, North and South America, Australia, and New Zealand (Ivens GM., 1979). Turkey has more than 133 genera and 1100 species of this family (Davis PH, 1965-1985). Chamomile is growing in different geographic regions of Turkey. Chamomile is extendable up to 25 centimeters long, flowering in April-September, an annual herbaceous plant. The leaves are finely divided and sessile. The middle part of the flower is yellow, the edges are white. It has been reported that harvesting in the summer , after dried it should be stored dry and moisture free places (Maranki, 2008).

Chamomile has been used in medical therapies for thousands of years, known in ancient Egypt, Greece, and Rome (Issac O, 1989). This plant has been considered by Anglo-Saxons as 1 of 9 religious plant given to human population by the God (Crevin JK, 1990). The chamomile medicine is kept in the pharmacological stores of 26 countries (Ompal Singh, 2011; Pamukov D, 1986). Chamomile is

available to use as a common source of healing in the world. It is used as sedative, anxiolytic, antiseptic, antispasmodic, anti-inflammatory, mildly sudorific and treating for skin infections (Krishna Murti, 2012; Mericli AH., 1990; Salamon, 1992a).

The hydro alcoholic extract of Chamomile stops the early developmental stages of cellular and viral RNA synthesis in polioviruses (Vikas et al, 2010; Vilaginès P, 1985). German Chamomile extracts with ethanol stops the growth of polio and herpes viruses (Krishna Murti, 2012; Suganda, 1983). Some scientists reported that when cancer cells and normal cells exposure with same dose of Chamomile extracts that are formed by water and methanol triggers apoptosis in cancer cells but there is no change in normal cells (Srivastava JK, 2007; Vikas et al, 2010).

Matricaria chamomilla is a typical herbal plant used for its medical therapies. It was used in folk medicine and is still used in modern medicine nowadays. It can be used to treat upper respiratory tract disorders and some other diseases in daily life of human population. Therefore *Matricaria chamomilla* is a significant curing plant (Jackson, 2001) .

Ecological Aspects

The following ecological aspects reported about Chamomile. They are harmless wild flowers so their seeds are distributed by different factors. They can be grown in large areas in nature (Royer, 1999). They can form dense colonies and some species can occupy more than 50% of the vascular plant species in an ecosystem (Royer, 1999). They like to live sunny places, slightly clayed and calcareous soils (T.C. M.E.B, 2008). German chamomile grows on all type of soil, on the other hand, growing the plant on rich, heavy, and damp soils should be avoided. Chamomile likes to live temperature ranging from 2°C to 20°C. (Ompal Singh, 2011)

Chemical Methods that are Used in Preparation of Chamomile Extracts.

Ethanol, dimethyl sulfoxide (DMSO), methanol, dichloromethane, petroleum ether, ethyl alcohol, hydroalcohol, water, gas etc. substances are solvents used during the preparation of chamomile extract. Air-dried and freezing methods are used in the preparation of Chamomile extracts. A. Raala et al. reported analysis of 5 different species of Asteraceae flowers oil by gas chromatography chemical analysis (SPB-5 and SW-10) and mass spectrometry methods in Estonia. Analyzed results are presented in the following tables (Ain Raala*, 2011).

Table 2.1 Chemical analysis of Chamomile oil (Ain et al, 2011).
Analysis methods 1- SPB-5, 2-SW-10, 3- Mass spectrometry

Chemical compound	**SPB-5**	**SW-10**	***Matricaria chamomilla***	**Identification Methods**
α-Pinene	927	1125	Tr	1,2,3
β-Pinene	969	1116	0.2	1,2,3

6-Methyl-5-hepten-2-one	984	1344	0.1	1,2,3
Myrcene	988	1168	0.1	1,2,3
n-Octanal	1002	1278	0.2	1,2,3
α-Terpinene	1012	1181	Tr	1,2,3
p-Cymene	1018	1272	0.2	1,2,3
Limonene	1023	1202	Tr	1,2,3
1,8-Cineol	1026	1208	0.2	1,2,3
(E)-β-Ocimene	1044	1254	0.2	1,2,3
γ-Terpinene	1054	1246	0.2	1,2,3
Artemisia ketone	1058	1353	0.8	1,2,3
2-Methylbutyl 2-methylbutyrate	1100	1300	0.2	1,2,3
n-Nonanal	1103	1400	0.2	1,2,3
Terpinen-4-ol	1172	1606	0.1	1,2,3
α-Terpineol	1187	1704	0.1	1,2,3
cis-3-Hexenyl isovalerate	1234	1454	Tr	1,2,3
α-Copaene	1367	1485	Tr	1,2,3
Decanoic acid	1398	2292	0.2	1,2,3
(E)-β-Caryophyllene	1408	1588	0.1	1,2,3
(E)-β-Farnesene	1455	1668	2.3	1,2,3
Alloaromadendrene	1464	1632	0.1	1,2,3
Germacrene D	1470	1696	0.2	1,2,3
α-Muurolene	1485	1725	0.2	1,2,3
Bicyclogermacrene	1490	1720	Tr	1,2,3
n-Undecanoic acid	1492	2350	0.2	1,2
Isofaurinone	1503	1900	0.2	1,2

δ-Cadinene	1510	1750	0.1	1,2,3
γ-Cadinene	1523	1752	0.1	1,2,3
NI (4), hotrienol structure, acetate?	1554	2035	Tr	1,2,3
(*E*)-Nerolidol	1563	2032	0.3	1,2,3
Dendrolasin	1563	2044	Tr	1,2,3
Spatulenol	1568	2120	**2.4**	1,2,3
Caryophyllene oxide	1572	1965	0.1	1,2,3
Dihydronerolidol	1580	2108	0.2	1,3
Viridiflorol	1595	2044	0.1	1,2,3
NI (8)	1600	2051	0.1	1,2,3
Geranyl isovalerate	1608	1924	0.3	1,2,3
Cubenol	1619	2100	0.1	1,2,3
γ-Eudesmol	1627	2157	0.3	1,2,3
γ-Cadinol	1635	2182	0.2	1,2,3
Bisabolol oxide B	1644	2125	**9.9**	1,2,3
α-Eudesmol	1646	2218	0.1	1,2,3
Alloaromadendrene epoxide	1657	2226	Tr	1,2,3
Bisabolone oxide A	1675	2163	**13.9**	1,2,3
α-Bisabolol	1688	2215	**5.6**	1,2,3
Geranyl tiglate	1700	2184	0.5	1,2,3
Chamazulene	1713	2370	**4.7**	1,2
Bisabolol oxide A	1748	2421	**39.4**	1,2,3
Myristic acid	1773	2713	0.1	1,2,3
n-Octadecane	1800	1800	0.2	1,2
Hexahydrofarnesyl acetone	1842	2160	0.1	1,2,3

Table 2.1- Continue **-** Chemical analysis of Chamomile oil (Ain Raala*, 2011).

Chemical compound	**SPB-5**	**SW-10**	***Matricaria chamomilla***	**Identification Methods**
(*E*)-En-yne-dicycloether, MW200	1882		0.4	1,2,3
n-Nonadecane	1900	1900	0.5	1,2,3
(*Z*)-En-yne-dicycloether, MW214	1933	-	0.4	1,3
Palmitic acid	1975	2920	Tr	1,2,3
n-Eicosane	2000	2000	0.1	1,2,3
γ-Palmitolactone	2100	-	0.1	1,3
cis-Linoleic acid	2120	-	0.1	1,2,3
n-Tricosane	2300	2300	0.1	1,2,3
n-Tridecanal	1500	1795	Tr	1,2,3
Compound groups				
Monoterpenes			0.9	
Oxygenated monoterpenes			1.5	
Sesquiterpenes			3.1	
Oxygenated sesquiterpenes			73.4	
Polyacetylenes			12.3	
Aliphatic acid and esters			0.7	
Other compounds			6.6	
Not identified			0.1	
Total			98.6	
Oil volume, %			0.15	

NI:Non Isomer, tr: traces (< 0.05%),

According to result of chemical analysis of 5 different species of Asteraceae flowers oil 115 compounds analyzed in the studied samples, which assumed for 49.1–98.5% of the total amount of oil (Ain Raala*, 2011). The results of chemical analysis showed that most abundant compounds in chamomile oil are bisabolol oxide A, bisabolone oxideA, (Z)-en-yne-dicycloether, bisabololoxide B, α-bisabolol, and chamazulene (Ain et al, 2011). According to results of five different species flower oil of Asteraca family 14 common chemical compounds identified (Ain et al, 2011).

4. Pharmacological Properties of Chamomile

The chamomile has many chemical compounds that contain pharmacological effects. The main active components of chamomile oil are Chamazulene, Apigenin and Bisabolol (Gardiner, 1999). Chamazulene has stopped leukotriene synthesis in neutrophil, have got antioxidant activity (Gardiner, 1999; Safayhi, 1994). 50% Bisabolol German chamomile consist of essential oil and clears spasm in smooth muscle in intestine (Achterrath-Tuckermann, 1980; Forster, 1980) also it has got antibacterial, anti-inflammatory, pain relief, ulcer-protective and antifungal effects. (Achterrath-Tuckermann, 1980; Berry, 1995; Gardiner, 1999). Flavonoids, apigenin and luteolin are responsible for anti-inflammatory effect, eliminating gas and spasm (Salamon, 1992). The presence of apigenin bounds to GABA receptors, which causes calming effect in humans (Gardiner, 1999; Salamon, 1992a; Viola, 1995).

4.1. Anti inflammatory Effects of Chamomiles

Matricaria chamomilla is an aromatic and medicinal plant with antioxidant,anticancer, antigen toxic, anti-inflammatory, antimicrobial and neuroprotective activities (Lim, 2014). Bisabolol compound has been found to reduce inflammation, fever and joint disorders (Isaac, 1979; Krishna, 2012). Studies were conducted in animals showed that apigenin has got anti-inflammatory effect(Isaac, 1979; Krishna, 2012).

4.2. Antimicrobial and Antiviral Effects of Chamomiles

Chamomile has some chemical compounds that have antimicrobial and anviral effects. Chamomile oil helps in healing of ear infections (acute otitis) due to effect on 3 subspecies of *Staphylococcus aureus* and Candidas (Nogueira, 2008; Vikas, 2010).

Chamomile oil has been found to be a candidate that can be used in therapy as agents in herpes genitalis disorders (Koch C, 2008). Hydro alcoholic extract of chamomile oil has stopped the early developmental stages of cellular and viral RNA synthesis of poliovirus (Vikas, 2010; Vilaginès, 1985). Extracts which is consist of German chamomile and ethanol, was stopped the growth of herpes virus and polio virus (Krishna, 2012; Suganda, 1983).

One of the components of Chamomile oil is a alpha-bisabolol, which was identified that has strong effect against component of gram positive and gram negative bacteria (Kedzia, 1991; Krishna, 2012). Chamazulene has potent antimicrobial activity (Kedzia, 1991; Krishna, 2012). Spiroeter has weak

activity against gram-positive, while strong activity against gram negative (Kedzia, 1991; Krishna, 2012). Mexican daisy(*Tridax procumbens*) has got potential presence of antimicrobial activity opposite to *Staphylococcus aureus* and *Escherichia coli* bacteria (G. Thilagavathi, 2007). Chamomile and tea tree oil is used in the elimination of various stains (Sadr, 2006; Vikas, 2010). Antiviral effects of chamomile extract was patented in Russia by patent number 2311194 (Buryakova, 2007).

5. Cytotoxic Activity of Chamomile Extracts

Cytotoxicity assays (cell viability) are mostly used by the pharmaceutical industry to monitor for cytotoxicity in compounds of medical plants. Microculture tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxic assay was utilised to determine the cancer (HeLa CEACAM) cell viability after addition of extracts or compounds. This assay is depend on the ability of the dehydrogenase enzymes in surviving cells to change soluble yellow MTT (into insoluble purple formazan) (Mosmann, 1983). The assay was carried out in the Laboratory of Medical Biology and Genetics, Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University.

6. Material and Methods

6.1. Preparation of Plant Extracts:

100 gram Air-dried chamomile flowers were measured into 250ml Erlenmeyer beaker and 200 ml of ethyl alcohol were added to the samples and the suspension was stirred slightly. After addition of ethyl alcohol Chamomile extract were stayed one day in a dark room at room temperature. Filtering of the extracts were done by Whatman filter paper and ethanol was removed by rotary vacuum evaporator at 80 ºC . After filtration through filter paper, the debris was re-extracted twice, and then the combined extracts of every sample were evaporated in evaporator. After complete removal of ethyl alcohol chamomile extracts was made ready for use by dissolving in sterile distilled water.

6.1.1. Cultivation of HeLa CEACAM Cells

Cells, virus and time-course analysis of MHV infection HeLa-CEACAM (Ulasli, et al., 2014; Verheije et al, 2008) that were used to propagate and titrate MHV-A59 (mouse hepatitis virus–A59) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma,St. Louis, MO) containing 10 % fetal calf serum (Thermo,Waltham, MA), 100 IU of penicillin/ml and 100 lg/ml of streptomycin (both from Life Technologies, Rochester,NY). HeLa-CEACAM cells were inoculated with MHVA59 at a minute of 30 (Ulasli, et al., 2014; Ulasli M, 2010; Verheije et al, 2006). After 30 min, the infected cells were washed and maintained in complete medium. Subsequently, the infected cells and culture supernatants were collected for analysis at 0, 6, 8 hours post infection. When plant extracts were poured to the HeLa-CEACAM cells, they were poured after viral infection and were washed away 1 hour later. Cells are counted on Thoma slide and they (1×10^4 cells per each well) were implanted into 24-well micro culture plates and allowed to observe for 24 hours. Then fresh growth medium was poured onto the cells. Then we waited for getting confluence of 70% of the cultivated cells in each micro culture plate.

6.1.2. Adding of Chamomile Extracts onto the Cells.

Different concentrations ratio such as 1/10, 1/50, 1/100, 1/1000 were calculated for *Matricaria chamomilla* extracts . After getting confluence of 70% of the cultivated cells in each micro culture plate. Then, each HeLa CEACAM cell line was exposed to extracts at 10, 50, 100,1000 µg/ml Chamomile extract concentrations for 24 hours and 48 hours. Viability was measured by MTT assay.

6.2. Cell Viability Test by MTT Assay

The cytotoxic effects of *Matricaria chamomilla* extracts on HeLa CEACAM cells was measured by MTT (3- (4,5-dimethylthiazol-2-yl) -difeniltetrazoliumbromid) assay. Medium of micro culture cell was removed after 24 and 48 hours and 500 µl (ml/mg) MTT agent was added in each plate. Micro culture cells was checked under microscope and was incubated 1hour at 37° C in incubator that containing 5% CO_2 under dark condition. Then 500 µl Dimethyl Sulfoxide (DMSO) was added in each well and was evaluated by the spectrophotometer (Biotek, USA) at 570 nm. MTT operation was repeated twice.

7. Results

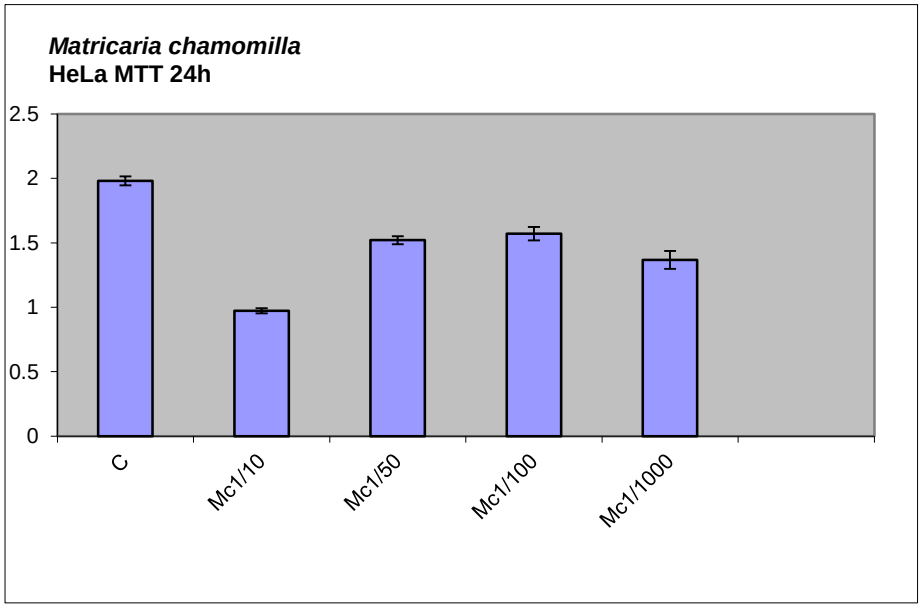

Figure 1. Effects of Chamomile extracts on the survival of HeLa CEACAM cells. HeLa CEACAM cells were incubated with the diluted extracts for 24 hours and cell toxicity was tested by MTT assay. C is control.

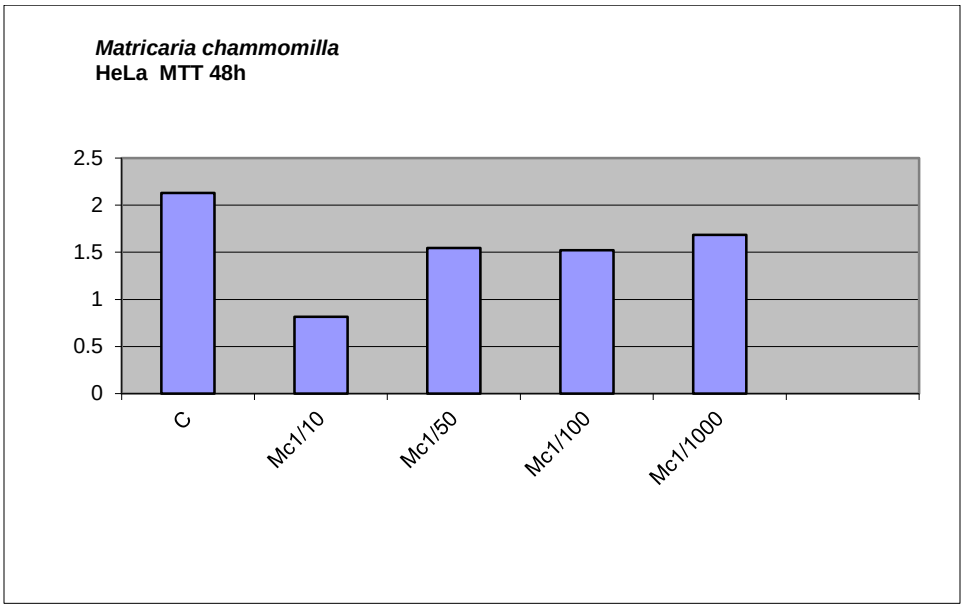

Figure 2. Effects of *Matricaria chamomilla* extracts on the survival of HeLa CEACAM cells. HeLa CEACAM cells were incubated with the diluted extracts for 48 hours and cell toxicity was measured by MTT assay. C is control.

In order to determine any possible toxic effects of *Matricaria chamomilla* extracts on HeLa CEACAM cells, serial solutions (1/10, 1/50, 1/100, 1/1000,) of *Matricaria chamomilla*, extracts were incubated with HeLa CEACAM cells for 24 and 48 hours. The cell viability was measured by the MTT assay. *Matricaria chamomilla* extracts showed toxic effect at 1/10 solution, however toxicity of the extracts definitely reduced at 1/50 solution for 24 and 48 hours (**Figures. 1, 2**). These results suggest that the cytotoxic effect of *Matricaria chamomilla* extracts were concentration dependent but not time-dependent in that less cells were viable at 1/10 solution compared to 1/1000 solution. According to results, the plant extract solution of 1/1000 was calculated as an effective dose.

8. Conclusion

There are many publications about pharmacological properties of *Matricaria chamomilla*. In this article I tried to demonstrate pharmacological properties and cytotoxic effect of *Matricaria chammomilla* extracts with ethyl alcohol. Nowadays, one of the most popular topic of researches is study with cancer cells. Many plant extracts used in folk medicine and in modern medicine. Studying cytotoxic effect of plants extracts is one of the basic step of cancer researches. The main aim of this kind of researches is to find medical treatment against cancer cells development. In this study just I tried to find active dose of *Matricaria chamomilla* extract for future researches.

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References

Achterrath-Tuckermann U, K. R., Flaskamp E, Isaac O, Thiemer K. (1980). Pharmacological investigations with compounds of chamomile. V. Investigations on the spasmolytic effect of compounds of chamomile and Kamillosan on the isolated guinea pig ileum. *Planta Medica, 39*, 38-50.

Ain Raala*, H. K., Anne Oravb, Elmar Araka, Tiiu Kailasb, and Mati Müüriseppb. (2011). Content and composition of essential oils in some Asteraceae species. *Proceedings of the Estonian Academy of Sciences, 60*(1), 55-63. doi: doi: 10.3176/proc.2011.1.06

Berry, M. (1995). Herbal products. Part 6. Chamomiles. . *Pharmaceutical Journal, 254*, 191-193.

Buryakova, I. V., Kurilova, A. I., Badytchik, L. I., andZamarenov, N. A. (2007).

Crevin JK, P. J. (1990). *Herbal medicine past and present.* USA: Duke University Press.

Davis PH. (1965-1985). *Flora of Turkey and East Aegean Islands.* Edinburgh: Edinburgh University Press.

Forster HB, N. H., Lutz S. . (1980). Antispasmodic effects of some medicinal plants. *Planta Medica, 40*, 390-319.

Gardiner, G. (1999). Chamomile (Matricaria recutita, Anthemis nobilis). from The Longwood Herbal Task Force,The Center for Holistic Pediatric Education and Research

Isaac, O. (1979). Pharmacological investigations with compounds of camomile, I. On the pharmacology of - (-)alpha-bisabolol and bisabolol oxides. *Planta Med., 35*, 118-124.

Issac O. (1989). *Recent progress in chamomile research- medicines of plant origin in modern therapy.* Czecho-Slovakia: Prague press.

Ivens GM. (1979). Stinking mayweed. *N Z J Agric, 21*(3), 138.

Jackson, T. (July, 2001). Medical Attributes of Matricaria chamomilla - Chamomile

Kedzia, B. (1991). Antimicroorganism`s activity of Oil Cammomillae and its components. *Herba Polonica, 37*, 29-38.

Koch C, R. J., Schneele J, Schnitzler P. . (2008). Inhibitory effect of essential oils against herpes simplex virus type 2. *Phytomedicine, 15 (1-2)*, 71-78.

Krishna Murti, M. A. P., Vipul Gajera and Jinal Solanki,. (2012). Pharmacological properties of Matricaria recutita: A Review. *Pharmacologia, 3(8)*, 348-351.

Lim, T. (2014). Matricaria chamomilla. Edible Medicinal And Non-Medicinal Plants. . *Springer.*

Ahmet Maranki. (2008). *Kozmik bilgiler ışığında Şifalı Bitkiler*: Mozaik yayınları.

Mericli AH. (1990). The lipophilic compounds of a Turkish Matricaria chamomilla variety with no chamazuline in the volatile oil. . *Int J Crude Drug Res. , 28*(145), 7.

Mosmann T. (1983). Rapid colorimatric assay for cellular growth andsurvival: Application to proliferation and cytotoxicity assays. *J.Immunol. Methods, , 65*, 55-63.

Nogueira JC, D. M. F., Lima EO. . (2008). In vitro antimicrobial activity of plants in Acute Otitis Externa. *Braz J Otorhinolaryngol, 74(1)*, 118-124.

Ompal Singh, Z. K., 1 Neelam Misra, and Manoj Kumar Srivastava. (2011). Chamomile (Matricaria chamomilla L.): An overview. *Pharmacogn Rev. , 5(9)*, 82–95.

Pamukov D, A. C. (1986). *Natural pharmacy (in Slova).* (1st ed ed.). Bratislava: Priroda.

Renuka, C. (Ed.). (1992). *Rattans of the Western Ghats: A Taxonomic Manual. .*

Royer, F. a. R. D. (1999). Weeds of the Northern US and Canada. (Germplasm Resources Information Network - (GRIN) 2004). Retrieved USDA, ARS, National Genetic Resources Program., from University of Alberta Press

Sadr Lahijani MS, R. K. H., Heady R, Yazdani D. . (2006). The effect of German chamomile (Marticaria recutita L.) extract and tea tree (Melaleuca alternifolia L.) oil used as irrigants on removal of smear layer.a scanning electron microscopy study. *int Endod J, 39(3)*, 190-195.

Safayhi H, S. J., Sailer ER, . (1994). Ammon HPT. Chamazulene: An antioxidant-type inhibitor of leukotriene B-4 formation. *Planta Medica, 60*(410-413).

Salamon, I. (1992a). Chamomila: A medicinal plant. Herb. *Spice Med. Plant digest,, 10*, 1-4.

Salamon I. (1992). Chamomile, A Medicinal Plant. The Herb, Spice, and Medicinal. *Plant Digest, 10*, 1-4.

Srivastava JK, G. S. (2007). Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. . *J Agric Food Chem., 55(23)*, 9470-9478.

Suganda, A. G., M. Amaros, L. Girre, and B. Faucconnier. (1983). Inhibitory effects of some crude and semi-purified extracts of France on multiplication of human herpes virus I and Poliovirus 2 in the cell culture. *J. Nat. Prod., 46*, 626-632.

T.C. M.E.B. (2008). *Bahçecilik Compositae familyası*. Ankara.

Thilagavathi, S. K. B. (2007). <microencapsulation of herbal extracts for.pdf>. *Indian journal of Fibre and Textile research, 32*, 351-354.

Ulasli M, V. M., de Haan CA, Reggiori F (2010). Qualitative and quantitative ultrastructural analysis of the membrane rearrangements induced by coronavirus. *Cell Microbiol, 12*, 844–861.

Ulasli, M. S. A. G. R. B. O. Y., Cakmak, S. O. M. I. Y. Z. I. E. A., & Ahmet Arslan. (2014). The effects of Nigella sativa (Ns), Anthemis hyalina (Ah) and Citrus sinensis (Cs) extracts on the replication of coronavirus and the expression of TRP genes family. *Mol Biol Rep, 41:1703–1711*. doi: DOI 10.1007/s11033-014-3019-7

Verheije MH, R. M., Mari M, Te Lintelo EG, Reggiori F,, & van Kuppeveld FJ, R. P., de Haan CA, . (2008). Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. *PLoS Pathog 4:e1000088*.

Verheije MH, W. T., van Beusechem VW, de Haan CA,, & Gerritsen WR, R. P. (2006). Redirecting coronavirus to a nonnative receptor through a virus-encoded targeting adapter. *J Virol, 80*, 1250–1260.

Vikas, G. P. M., Parveen Bansal1, Sukhbir L Khokra3, Dhirender Kaushik3. (2010). Pharmacological Potential of Matricaria recutita-A Review. *International Journal of Pharmaceutical Sciences and Drug Research, 2(1)*, 12-16.

Vilaginès P, D. P., Vilagines R. . (1985). Inhibition of poliovirus replication by an extract of Matricaria chamomilla (L). *C R Acad Sci III., 301(6)*, 289-294.

Viola H, W. C., Levi De Stein M, et al. . (1995). Apigenin, a component of Matricaria recutita flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. *Planta Medica, 61*, 213-216.