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ANTIBACTERIAL AND ANTICARCINOGENIC EVALUATION OF COMMERCIAL CALENDULA OFFICINALIS AND TARAXACUM OFFICINALE

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ABSTRACT: Public interest on herbal drugs increase every passing day in both developed and developing countries. However, its regulations more strict in developed countries than in developing countries. Clarifying the effectiveness and adverse effects of this product is becoming more important. Calendula officinalis and Taraxacum officinale both commonly using in herbal-based therapies due to the speculated antibacterial and anti-cancer effects of them. Several effects of these plants have been associated with their ingredients, such as phytochemicals, flavonoids, essential oils, etc. In this study, we aimed to investigate antibacterial effects and potential anticancer effects of commercial Calendula officinalis and Taraxacum officinale products obtained from herbalist. Antibacterial effects of methanolic extracts of Calendula officinalis and Taraxacum officinale were evaluated via microbroth dilution technique using the Clinical Laboratory Standards Institute (CLSI) recommendations. Anti-cancer effects were evaluated with the MTT cytotoxicity test on the human neuroblastoma cell line (SH-SY5Y). Our results indicate that commercial products show lower antibacterial effects separately and with 1:1 combination that was reported in previous different studies. It has been observed that both methanolic plant extracts were shown 100% viability in desired concentrations.

INTRODUCTION: Global usage of herbal products that include nutraceuticals and herbal medicines increases rapidly for the purpose of different cure types of diseases. Obtaining these products is very easy in developing countries. Most people in developing countries believed that herbal products as effective as a medical cure.



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However, in developed countries, herbal products under strict control. Even though these strict control protocols in developed countries, people embraced these types of remedies commonly. Due to several recent herbal drugs come into the market with increasing rate, efficacity, safety, and toxicity, studies gain importance every passing day.

Most herbal products come into the market without enough safety evaluation, and people use this product by themselves without expert prescriptions. Misuse of herbal products causes a wide range of adverse effect as allergy, liver, and other target organ toxicity, even death ¹⁻⁵.

Callendula officinalis, Fig. 1A includes different types of phytochemicals as carotenoids, terpenoids, flavonoids, quinones, etc. that are used in herbal medicine for the cure. It has been speculated that C. officinalis exert anti-inflammatory, anti-bacterial and anti-fungal, antioxidant, hypoglycemic and gastroprotective, antidiabetic, anti-HIV, and anti-cancerous effects ⁷⁻¹⁵. *C. officinalis* use for the treatment of abrasions, burns, ulcers, inflammations, eczema, and wounds due to its antibacterial and anti-fungal effects. Different parts of the plant exert different effectiveness. Flower extracts Show higher antibacterial activity. however, reproductive parts of plants have lesser antibacterial activity. It has been reported that especially methanolic extract of the flower include great antibacterial activity against gram-negative and gram-positive bacteria 7. C. officinalis have antifungal effects on different candida species ⁶. Taraxacum officinale Fig. 1B, generally used in salads and vegetable foods. T. officinale include sesquiterpenes, lactones, glycoisdes, free sterols taraxerol), polysaccharides, (taraxasterol and pectin, resin and mucilage and various flavonoids. T. officinale generally used for liver and kidney problems, edema, skin problems, heartburn, and gastrointestinal problems. It has been speculated that T. officinale have Hypoglycemic effects, antyoxidant effect, diuretic, antibacterial and antiinflammatory effects ³. In this study, we aimed to investigate antibacterial and cytotoxic effects of commercial Calendula officinalis, Taraxacum officinale products obtained from an herbalist.

MATERIALS AND METHODS:

Ethanolic Plant Extraction: 30 g *Calendula officinalis* and *Taraxacum officinale* commercial plants were weighed. 300 ml ethanol was added on separately added on plants and incubated for 24 h on a shaker. Ethanol was vaporized by distillation equipment. Dried extracts were stored at +4 °C.

Cytotoxicity Evaluation-MTT Test: The MTT assay was performed to cytotoxic effects of the

tested *Calendula officinalis* and *Taraxacum officinale* concentrations for each plant; $100 \mu g/ml$, $50 \mu g/ml$ $25 \mu g/ml$ $12,5 \mu g/ml$ and solvent control as DMSO (1% finally). Human neuroblastoma (SH-SY5Y) cells were seeded into 96-well plates (1×10⁴ cells/ 100 μ l of medium/well) and incubated overnight. After the treatment of *Calendula officinalis* and *Taraxacum officinale* for 24 h, the MTT solution was added into each well, and the cells were further incubated for 3 h at 37 °C in the dark. After incubation, the medium was discarded, and 100 μ l of DMSO was added to each well for dissolve formazan crystals. Optical density was measured at 570 nm by using a microplate reader (Biotek, Epoch, Vermont, USA).

Antibacterial Assay: Antibacterial activity of Calendula officinalis and Taraxacum officinale evaluated against Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Escherichia coli **ATCC** 25922. Klebsiella **ATCC** 4352, Pseudomonasa pneumoniae eruginosa ATCC 27853, Enterococcusfa ecalis ATCC 29212 ve Candida albicans ATCC 10231 by the micro broth dilution technique using the Clinical Laboratory Standards Institute (CLSI) recommendations. The MIC (minimum inhibitory values were concentration) determined comparison with standard agents.

RESULTS:

Cytotoxicity Assay: According to MTT test results, *Calendula officinalis* and *Taraxacum officinale* extract administration exerts 100% viability on SH-SY5Y cell lines **Table 1**.

Antibacterial Assay Results: According to antibacterial assay results, it has been observed that ethanolic extracts of commercial *Calendula officinalis* and *Taraxacum officinale* products exerted low antibacterial activity, additionally their 1:1 combination showed very low antibacterial activity **Table 2**.

TABLE 1: VIABILITY (%) RESULTS OF CALENDULA OFFICINALIS AND TARAXACUM OFFICINALE BY MTT TEST

Calendula officinalis (µg/ml)	Viability (%)	Taraxacum official (µg/ml)	Viability (%)
12,5	100	12,5	100
25	100	25	100
50	100	50	100
100	100	100	100

TABLE 2: ANTIBACTERIAL ACTIVITY RESULTS

	Taraxacum	Calendula officinalis	1:1 Combination	Reference (MIC)		
	officinale Starting Concentration:2000	Starting Concentration: 2000	Starting Concentration: 2000			
	μg/ml)	μg/ml)	μg/ml)			
(Pseudomonas aeruginosa)	62,5	500	250	Ceftazidime ^{2, 4}		
ATCC 27853				4.0		
(Klebsiella pneumoniae)	250	500	500	Cefuroxime-Na 4,9		
ATCC 4352						
(Escherichia coli) ATCC	125	500	500	Cefuroxime-Na 4,9		
25922				2.4		
(Enterococcus faecalis)	15,6	250	125	Cefuroxime-Na ^{2, 4}		
ATCC 29212				0.0		
(Staphylococcus	125	500	500	Cefuroxime-Na 9, 8		
epidermidis) ATCC 12228				1.2		
(Staphylococcus aureus)	62,5	62,5	125	Cefuroxime-Na 1, 2		
ATCC 29213				4.0		
(Candida albicans) ATCC	125	500	500	Clotrimazole 4,9		
10231						
MIC: minimun inhibitory concentration						

DISCUSSION: Several scientific studies on different therapeutic effects of several types of herbal drugs were published in scientific databases. Additionally, several speculative information's publishing on media for different herbal drugs. People could try different plants that were obtained from herbalists or they could harvest to cure themselves. In case, it is very important to clarify the adverse effects of herbal drugs to deliver accurate information. It has been reported that different calendula species have antibacterial effects on different microbes 11. In our study commercial products, methanolic extract was shown lower antibacterial activity. Taraxacum officinale' santibacterial effects were shown in different studies ⁸⁻¹⁶. In this study, *Taraxacum* officinale' methanolic extract was shown lower antibacterial activity, additionally 1:1 combination of these two plants also exerts lower antibacterial activity. Several different studies are focusing on anticancer effects of Calendula officinalis and Taraxacum officinale plant extracts ^{9, 10}. However, in our study, both of Calendula officinalis and Taraxacum officinale's 100 µg/ml, 50 µg/ml 25 μg/ml 12, 5 μg/ml methanolic extracts concentrations administration were shown 100% viability.

CONCLUSION: In conclusion, commercial *Calendula officinalis* and *Taraxacum officinale* products showed lower antibacterial effects separately and were not shown anti-cancer effects on the human neuroblastoma cell line. Further, *invivo* and *in-vitro* studies needed to clarify

antibacterial and anti-cancer effects of these plants with different extraction methods. It is very important to inform the public correctly about herbal product collection, extraction methods, and therapeutic drug forms for the therapy.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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