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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE LEAVES OF (Taraxacum Officinale) PLANT

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ABSTRACT

The development of antibiotic resistance has been required to discover the new antimicrobial agents mainly among plant extracts. In this study, the antibacterial and antifungal properties of *Taraxacum officinale* were investigated. The antibacterial activity of the extract was examined by agar dilution method and determined the minimum inhibitory concentration (MIC) value according to clinical and laboratory standards institute (formerly CLSI). The antifungal effect of the extract was examined with the broth macrodilution method according to CLSI. Ethanolic extract of *Taraxacum officinale* leaves described the highest activity against *B. cereus* (15.25 mg/ml) and *B.subtilis* (61 mg/ml) while it showed the lowest activity against *E. coli, S. enteritidis, P. aeruginosa, S. aureus, S. epidermidis* and *E. faecalis* (122 mg/ml). The extract was found to be effective against all strains of the fungal used but only in high concentrations. Extract demonstrated the highest effectiveness against *C. albicans* and *M. nanum* (610 mg/ml) although it indicated the lowest effectiveness against *C. krusei, C. parapsilosis, M. pachydermatis, M. canis, M. gypseum* and *T. mentagrophytes* (1220 mg/ml). As a conclusion, *Taraxacum officinale* can be exploited in the management of pathogenic bacteria and fungi to develop an alternative therapy.

Keywords: Antibacterial, Antifungal, Taraxacum officinale

1. INTRODUCTION

Traditional herbal medicine and remedies have played an essential role in the treatment of various diseases. Herbs have been used in worldwide medicinally as a source of many drugs (Mahesh and Satish 2008). It has been estimated that 25% of prescriptions are directly or indirectly of plant origin (Doughari 2006). Plant medicine is used by most of the world cultures, primarily in developing countries, because of better cultural acceptability, better adaptation to the human body and fewer side effects than standard drugs (Oseni and Yussif 2012). According to World Health Organization data, nowadays, 80% of the world's population continues to rely on traditional medicines for health care(Shütz et al.2006).

Due to the unusual genetic capacity of bacteria, overuse of antibiotics by humans, transfer of resistance genes, clinical, agricultural or otherwise antibiotic application, the antimicrobial resistance mechanisms have developed. Antimicrobial resistance in medicine is currently

Vol. 3, No. 06; 2018

ISSN: 2456-8643

recognised as an important public health problem by the World Health Organization (WHO) and various other national authorities. It confronts human and veterinary medicine with a major challenge of global dimensions to the possibility of therapeutic failure for life-saving treatments (Woods-Panzaru et al. 2009). In recent years, because of the unacceptable side effects of some antibiotics and the resistance, it has been required to discover the new antimicrobial agents especially mainly based on plant extracts (Bonjar 2004). However, although the traditional plants have been known to have antimicrobial activity since ancient times, there have been relatively few researches that investigate the efficacy of medically important bacterial and fungal (Woods-Panzaru et al. 2009).

Taraxacum officinale (often simply called "dandelion") is a flowering herbaceous perennial plant of the family *Asteraceae*. This plant grows in temperate regions of the world and is found mostly on the roadside, on scattered banks, in lawns, on shores of waterways and other areas with moist soils (Sohail et al. 2014). Dandelion was first noticed in the works of the Arabian physicians of the tenth and eleventh centuries. Throughout the ages, *Taraxacum officinale* has been known for its curative properties, various health-related benefits, and has been attributed to the use of extracts or plant itself for the treatment of different illness (Shütz et al. 2006). Dandelion had been used in folk medicine for the treatment of hepatitis disorders, inflammation and various gynaecological diseases such as breast and uterine cancers. The root is considered a diuretic and bitter digestive stimulant. (Amin et al. 2016, Williams et al. 1996). Preclinical studies on dandelion have revealed many features including an inflammation modulator, insulin stimulant, prebiotic, immunomodulator, antiangiogenic, laxative, anti-rheumatism, choleretic, anti-carcinogen, and hypoglycemic activities (Shütz et al. 2006).

Traditionally, in different regions of the world, *Taraxacum* is consumed as food in various forms. The roots are used as a coffee, the leaves are eaten cooked or raw like soups and salads, and they can be used in the preparation of tea. The leaves possessed a higher content of β -carotene, iron (Fe), calcium (Ca) and recommended as a natural source of vitamin C (Ivanov et al. 2018). It has been widely used in traditional medicine in Turkey as a spice or herb for a long time (Sengül et al. 2009). Given the potential of plants as a drug source for antimicrobial agents, many studies were conducted to screen the antibacterial activity of *Taraxacum officinale* (Sohail et al. 2014).

While the majority of reports on antimicrobial activity focus on antibacterial effects, there are few reports on antifungal effects. Fungal diseases may not be as common as other microbial infections, but when present, it is difficult to treat especially in immunocompromised individuals (Khan et al. 2011). Diseases of animals carried by animal vectors to man, and vice versa, are essential aspects of dermatology. The most common zoonotic dermatoses of dogs and cats are dermatophyte infections which constitute one of the most important groups of fungal infections in the world (Scott and Horn 1987). *Microsporum canis, M. gypseum* and *Trichophyton mentagrophytes* are the principal agents of dermatophytosis (Kanbe et al. 2003). These fungi are zoonoses, caused the infectious of keratinised tissues such as nails, hair and the skin of domestic animals, and called dermatophytoses (Moriello et al. 2017). To our knowledge; no studies are documenting the effectiveness of *Taraxacum* against dermatophytes.

Vol. 3, No. 06; 2018

Therefore, the purpose of the present study was to investigate the antibacterial and antifungal properties of the leaves of *Taraxacum officinale* plant against pathogenic bacteria and fungi that cause infection in humans and animals.

2. MATERIAL-METHOD

2.1 Herbs

Aerial parts (leaves) of a wild growing population of *Taraxacum officinale* plant were collected from non-forested parts of the north-facing slopes of the Trabzon province Çarşıbaşı district.

2.2 Extraction

After the type identification was made, they were allowed to dry under a shaded area for 20 days. Then, the herb materials were powdered by the grinder. The powdered herb material was (20 gr) extracted with 500 ml ethanol in soxhlet apparatus for 3h. It was filtered from Whatman No.1 filter paper. The filtrate extracts were concentrated under reduced pressure at 40°C, using a rotary evaporator (Heidolph, Laboratory 4000 series). The dried crude concentrated extracts were stored in a refrigerator ($+4^{\circ}C$) until used for analyses.

2.3 Antibacterial activity

The antibacterial activity of the leaf extract was examined by agar dilution method and determined the minimum inhibitory concentration (MIC) value according to clinical and laboratory standards institute (formerly CLSI) (CLSI 2012). The antimicrobial activities were evaluated against Gram-positive (*Staphylococcus aureus*, ATCC 29213, *Staphylococcus epidermidis*, ATCC 12228, *Enterococcus faecalis*, ATCC 29212, *Bacillus cereus*, ATCC 11778, *Bacillus subtilis*, ATCC 6633,) and Gram-negative (*Escherichia coli*, ATCC 25922, *Klebsiella pneumoniae*, ATCC 4352, *Pseudomonas aeruginosa*, ATCC 27853, *Salmonella enteritidis*, KUEN 349) bacteria. The strains were provided by the Faculty of Veterinary Medicine, Department of Microbiology Culture Collection, Istanbul University Cerrahpasa.

The leaf extract was dissolved in a 1 ml volume of DMSO. For two-fold step, ten serial dilutions of the extract between 0.2382 mg/ml - 122mg/ml were prepared with CAMBH in sterile tubes. The MIC value was detected as the lowest concentration of the extract gave complete inhibition of visible growth. Gentamicin sulphate (Sigma G1272) was used as the control antibiotic. The experiments were conducted twice, and data were averaged.

2.4 Antifungal activity

The antifungal effect of the leaf extract was examined with the broth macrodilution method according to CLSI (CLSI 2008). The antifungal activities were evaluated against yeasts (*Candida albicans, Candida parapsilosis, Candida krusei* and *Malassezia pachydermatis*) and fungi (*Microsporum canis, Microsporum gypseum, Microsporum nanum* and *Trichophyton mentagrophytes*). The strains were provided by the Faculty of Veterinary Medicine, Department of Microbiology Culture Collection, Istanbul University Cerrahpasa. Fluconazole (Sigma <u>PHR1160</u>) was used as the control antimicotic. The lowest extract concentration that completely inhibits the reproduction and can be determined with the naked eye was established as the MIC value. The tests were duplicated, and data were averaged.

Vol. 3, No. 06; 2018

3.RESULTS

The leaves of *Taraxacum officinale* plant were tested to determine their antibacterial activity against Gram-positive and gram-negative bacteria through the agar dilution method and the antifungal effect with the broth macrodilution method.

Ethanolic extract of *Taraxacum officinale* leaves was detected to demonstrate effectiveness in different concentrations against all strains (except *K. pneumoniae*) of the bacteria used. The extract showed the highest activity against *B. cereus* (15.25 mg/ml) and *B. subtilis* (61 mg/ml) while it demonstrated the lowest activity against *E. coli, S. enteritidis, P. aeruginosa, S. aureus, S. epidermidis* and *E. faecalis* (122 mg/ml). It was observed that the extract has no activity on *K. pneumoniae*.

The extract was found to be effective against all strains of the fungal used but only in high concentrations. They demonstrated the highest effectiveness against *C. albicans* and *M. nanum* (610 mg/ml) while it exhibited the lowest effectiveness against *C. krusei*, *C. parapsilosis*, *M. pachydermatis*, *M. canis*, *M. gypseum* and *T. mentagrophytes* (1220 mg/ml).

The results regarding the in vitro antibacterial and antifungal activity of the *Taraxacum officinale* leaves extract with MIC values are presented in Table I and II.

Table I. Antibacterial activity of *Taraxacum officinale* Leave extract and positive control

Bacteria	Taraxacum officinale (mg/ml)	Gentamycin (µg/ml)
Bacillus cereus	15.25	-
Bacillus subtilis	61	-
Enterococcus faecalis	122	4 - 16
Escherichia coli	122	0.25 - 1
Klebsiella pneumoniae	-	-
Pseudomonas aeruginosa	122	0.5-2
Salmonella enteritidis	122	-
Staphylococcus aureus	122	0.12 - 1
Staphylococcus epidermidis	122	-

(-); MIC value was not detected in the test concentrations

Table II. Antifungal activity of the Taraxacum officinale Leave extract and positive control

Vol. 3, No. 06; 2018

ISSN: 2456-8643

Fungal agents	Taraxacum officinale (mg/ml)	Fluconazole (µg/ml)
Candida albicans	610	0,06 - 2
Candida krusei	1220	0,125 - 2
Candida parapsilosis	1220	0.25 - 1
Malassezia pachydermatis	1220	0,06 - 2
Microsporum canis	1220	16-128
Microsporum gypseum	1220	16-128
Microsporum nanum	610	16-128
Trichophyton mentagrophytes	1220	16-128

4. DISCUSSION

In many countries of the world, there are many studies about the use of regional plants as the antimicrobial and antifungal drugs. Various authors have examined the antimicrobial and antifungal activity of herbs extracted by different methods.

Oseni and Yussif (2012) investigated the efficiency of aqueous and ethanol extracts of T. *officinale* leaf by agar diffusion method against E. *coli*, K. *pneumoniae*, P. *aeruginosa* and S. *aureus*. At the lowest concentration (50mg/ml), the ethanolic leaf extract inhibited only E. *coli*, and no zones of inhibition were shown against P *.aeruginosa* and K. *pneumoniae*. At the conclusion, they suggested that the antibacterial activity of ethanol and aqueous extracts is dose dependent and the ethanolic *Taraxacum officinale* leaf extract may be very useful in the discovery of new antibacterial agents.

In a study conducted by Jassim et al. (2012), the activity of *T. officinale* leaf in watery and ethanolic extracts (0.5 and 1 mg/ml) was examined against *E. coli, P. mirabilis* and *S. aureus* by agar diffusion assay. It has been found that the concentration 0.5 mg/ml was effective on *S. aureus* for both extracts and the ethanol extract was more active on than the aqueous extract

Similar results have been reported in another study where the ethanol extracts of leaves exhibited bactericidal activity using the broth microdilution method against *E. coli* and *S. abony* strains, while it was ineffective against *S. aureus* (Ionescu et al. 2013). Kenny et al. (2015) presented the antimicrobial activities of ethanol and water extracts as minimum inhibitory concentrations (MIC) against three Gram-positive (*S. aureus, MRSA, B. cereus*) and two Gram-negative (*E. coli* and *S. Typhimurium*) bacteria. They indicated that the ethanol extracts demonstrated antimicrobial effectiveness against *S. aureus, MRSA* and *B. cereus;* however, it was not active against *E. coli* and *S. Typhimurium*.

Vol. 3, No. 06; 2018

ISSN: 2456-8643

The *in vitro* antibacterial activity of water, methanol, hexane, chloroform, ethyl acetate and dichloromethane extracts of a different part of T. officinale(stem, root, and flower) were assessed against different pathogenic bacterial strains except for ethanol extract (Sharifi-Rad et al. 2018). Sengül et al. (2009) screened crude extracts from Taraxacum officinale for in vitro antimicrobial properties. The antimicrobial activity of the aqueous and methanol extracts was carried out by disc diffusion method. The methanol extract was proved to possess a considerable antimicrobial potentiality against 11 out of 32 microorganisms. The inhibition zone (6 mm) against Bacillus cereus was the highest inhibition zone. Sohail et al. (2014) observed that the methanol and chloroform extracts of T. officinale leaf were effective against P. aeruginosa, E. coli, S. aureus, B. subtilis and Micrococcus luteus while the extracts in D.H₂O did not show significant inhibition. Amin et al. (2016) indicated that the root methanol extract showed the highest antimicrobial potential followed by the ethyl acetate extracts. Khan et al. (2011) focused on the antimicrobial activities of the methanolic extract of *T. officinale* flowers. They demonstrated that extracts were effective against M. luteus, and V. cholerae but they did not show any activity against E. coli, P .aeruginosa, K. pneumoniae, B. subtilis, S. aureus, E. faecalis and Enterobacter. Moreover, they indicated that further researches are needed to explore the bioactive compounds. In another study, methanolic extracts show activity on S. aureus, P. aeruginosa, B. cereus, Shigella sonnei, S. enterica serovar Typhimurium, E. coli, K. pneumoniae, C.albicans, and C. neoformans (Fabri et al. 2011).

Ghaima et al. (2013) studied the antibacterial activities of ethyl acetate extract of dandelion against *E. coli*, *S. Typhi*, *A. hydrophila*, *S. aureus* and *B. cereus*. The inhibition zones were ranged from 14-18 mm, and the highest inhibition zone was towards *B. cereus*. According to the several comparative studies, methanol extraction seems to be one of the most effective methods.

Conversely, Woods –Panzaru et al. (2009) tested the antimicrobial activity of Dandelion leaf and roots (*Taraxacum officinale*) extracts against the 34 microorganisms, including 24 bacteria and ten fungi by disc diffusion method. They indicated that no antimicrobial activity was observed with any bacterial or fungal pathogen for Dandelion leaf and roots.

In this study, it was detected that the ethanol extract of *Taraxacum officinale* leaves demonstrated the highest effectiveness against *B. cereus* (15.25 mg/ml) by agar dilution method. While it showed the lowest effectiveness against *E. faecalis, E. coli, P. aeruginosa, S. enteritidis, S. aureus* and *S. epidermidis* (122 mg/ml), it was observed that the extract has no activity on *K. pneumoniae* similar to Sengül et al. (2009) and Ghaima et al. (2013), the highest effectiveness was against *B.cereus*. Similar to our results, Ionescu et al. (2013) reported that the extract had low antimicrobial activity against *S. aureus* and *E. coli*. Also, the same effect against *K. pneumoniae* was observed with Oseni and Yussif (2012) and Khan et al. (2011).

The antifungal activity of the leaves of *Taraxacum officinale* plant is rarely explored. Gatto et al. (2011) suggested that *T. officinale* extract not show significant antifungal activity against *Aspergillus* and *Penicillium* species. Petropoulos et al. (2018) have also presented that MIC values of *Taraxacum* sp plant extracts were lower than those of positive controls (bifonazole and

Vol. 3, No. 06; 2018

ISSN: 2456-8643

ketoconazole) against *Candida* and *Aspergillus* species. Woods-Panzaru et al. (2009) and Fabri et al. (2011) indicated that no antimicrobial activity was observed with a fungal pathogen for Dandelion leaf and roots.

In this study, the ethanol extraction of the leaves of *Taraxacum officinale* was found to be effective against all strains of the fungal used but only in high concentrations. Extract demonstrated the lowest effectiveness against yeast such as *C. albicans, C. krusei and C. parapsilosis,* similar to Fabri et al. (2011) and Petropolous et al. (2018).

To our knowledge, no studies are documenting the effectiveness of *Taraxacum* against dermatophytes. In this study, the ethanol extraction of the leaves of *Taraxacum officinale* was found to be effective against *M. nanum*, *M. pachydermatis*, *M. canis*, *M. gypseum* and *T. mentagrophytes* but only in high concentrations. Despite low effectiveness, it may provide an alternative solution against antifungal drugs.

5. CONCLUSION

Taraxacum officinale has quite promising prospects with its revealed results. Thus, the studies on the subject should proceed. Numerous studies have been conducted across the world to create new active ingredients or drugs. There are many real activities in herbal entities. Future studies are needed to improve the quality and quantity of research on herbals so that they can be used more efficiently and more safely in the management of pathogenic bacteria and fungi.

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Vol. 3, No. 06; 2018

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Vol. 3, No. 06; 2018

ISSN: 2456-8643

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