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Research Article

Antibacterial Activities of Widely Spread *Taraxacum Officinale* Dandelion in Al-Qadmous, Syria as Potential Therapeutic Strategy for Antibiotic Resistant Bacteria

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Abstract

Medicinal plants perform an important role within the treatment of upper respiratory illness such as sore throat, cold and flu. The study aimed to highlight the biological significance of antimicrobial activities exhibited by the ethanolic extract of *Taraxacum officinale* (dandelion) leaves and roots against the bacterial strains. The leaves and roots of *T. officinale* where collected from Qadmous area, Tartous, Syria, and were extracted with ethanol and tested for their antimicrobial activities against the bacterial strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and the pathogenic strain of *Streptococcus pneumoniae* along with trimethoprim/sulfamethoxazole

(TMP/SMX) antibiotics (positive control). The root extracts exhibited the maximum inhibition against *S. aureus, P. aeruginosa* and *E. coli* with zone of inhibitions measuring 24mm, 17mm and 12mm respectively, in comparison to the leaves extracts that exhibited antibacterial activities against *S. pneumoniae* (11mm) and *P. aeruginosa* (14mm). Notably, TMP/SMX antibiotics were observed to have larger antibacterial activities against *E. coli* (31mm) and *S. aureus* (30mm). These findings stem the possibility of using the extracts of *T. officinale*, especially the root extract as a co-factor to antibiotics in order to eliminate multiple antibiotics resistant bacteria, especially *P. aeruginosa* and *S. aureus*.

List of Abbreviations

MRSA : Methicillin-resistant *S. aureus*
TMP/SMX : Sulfamethoxazole and Trimethoprim
TPC : Total phenolic components

Introduction

The World Health Organisation evaluated that up to 80% of the world population often uses conventional remedies [1], with more than 35,000 plant species are utilised as medicine to alleviate illness in several human customary communities [2]. *Taraxacum officinale.* L. (dandelion) is a perennial weed plant from the Asteraceae (Compositae) family mostly found in the temperate area of the Northern hemisphere and several parts of the world [3]. However, the plant has medicinal significance [4], and is widely used across Syria [5]. Noticeably, the roots of *Taraxacum officinale* are maximally used for medicinal studies, however in many cases the leaves and whole plant are also used [6]. *Taraxacum officinale* contains therapeutic properties because of the presence of phytochemicals such as tannins, flavonoids that exhibits healing activities and are widely studied in various areas of human health treatment [7-10]. Compounds such as tannins [11] and flavonoids are known as anticancer agents [12], and joint pain inhibitor [13,14] respectively. This plant contains high concentrations of potassium, which is crucial to the process of diuresis and calcium, and reduces the feeling of numbness in the limbs [15]. *Taraxacum* also has anti-inflammatory properties related to the compound taraxasterol and the prevention of heart disease by reducing cholesterol levels [16]. Various parts of *T. officinale* have been investigated for their antioxidant properties [17, 18], anti-quorum sensing activities [19], anti-inflammatory properties related to compound taraxasterol and the prevention of heart disease by reducing cholesterol levels [20,21].

Figure 1: *Syrian Taraxacum officinale plant depicting its different organs. Leaves have been used in our study as exhibited by a rectangle box*

Additionally, *T. officinale* plant has the ability to get rid of heat and toxins in humans, and thus, reduce swelling, inflammation, excessive production of urine and the flow of bile from the liver [22]. A previous study [23] has reported the effects of *T. officinale* extracts on oxidative stress, inflammation, and lipid profile in C57BL/6 mice fed atherogenic diet and therefore there could be a possibility that plant could reduce the risk of atherosclerosis in humans. Therapeutically, *T. officinale* relieves cold symptoms and sore throats during flu season. For example, a study [24] demonstrated how *T. officinale* extracts can inhibit both A/PR/8/34 and WSN (H1N1) influenza viruses by inhibiting viral nucleoprotein synthesis and polymerase activity. The authors further investigated the characterisation of the active compounds of the extracts and their specific mechanism against influenza virus. Apart from their medicinal importance, *T. officinale* is often used as nutritious food and beverage [6]. The leaves are used in the salads, sandwiches, tea and often cooked as vegetables. The roots are often substituted for coffee and flowers for wine and schnapps [6]. Moreover, a study [25] has shown that 100g of dry matter of *T. officinale* Weber (dandelion) contains a total dietary fiber 47.80g, ash 14.55g and proteins 15.48g.

The antimicrobial properties of *T. officinale* plant extracts have been reported earlier [26,27] and in particular the root's ethanolic and methanolic extracts was found to be more effective in suppressing the growth of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA) and *Bacillus cereus strains* [28,29].

At the other end, over dosage of antibiotics are often linked to side effects on hosts which involve hypersensitivity, immunosuppression and allergic reactions [30]. These rapid uses of antibiotics lead to the development of antibiotic resistant microbial strains, causing rapid failure of chemotherapeutics, significantly

influencing the use of biological bioactive molecules to alleviate the human infections [31]. Therefore, information about antimicrobial potentials of plant extracts on different microbial strains are important in order to evaluate their potentials as antimicrobial agents especially against multidrug resistant and pathogenic microbial strains [32-37].

Therefore, the present study investigated the *in vitro* assay of the antibacterial activity of ethanol extracts of leaves and roots of Syrian *T. officinale* plant against Gram-positive (*Staphylococcus aureus, Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria. To the best of our knowledge, this is the first study conducted on the antimicrobial activities of *T. officinale* plant extracts in Syria.

Materials and Methods

Sample Collection

Fresh leaves and roots of *T. officinale* were collected from across Al-Qadmous countryside in February, 2019 and was transported to the Department of Microbiology Al Andalus University, Tartous, Syria. The plant materials were surface-sterilised by washing with tap water, disinfected in 1% bleach [sodium hypochlorite (NaOCl)] for 3 - 5 min, rinsed with sterilised distilled water (dH_2O) for 2 min and then dried using laboratory tissue paper.

Plant Extract Preparation

All plant parts were air dried in shade for two weeks at room temperature 20-25°C followed by in an oven at 40°C for 15 minutes every day for a week until the stability of weight was attained. The dried leaves and roots were pulverized using a mechanical grinder. The plant extracts were prepared by cold extraction method using ethanol solvent [38]. Approximately 10g of each the powdered plant materials were soaked in 70mL of ethanol and left for 2 days at room temperature in order to diffuse out the secondary metabolites into the solvent. Further the solution was filtered with Whatman filter paper No. 2 (Munktell & Filtrak GmbH, Barenstein, Germany), and the filtrates were concentrated at 40°C for 3 days at 25 rpm on a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). The residue was collected and stored at 4°C until further used.

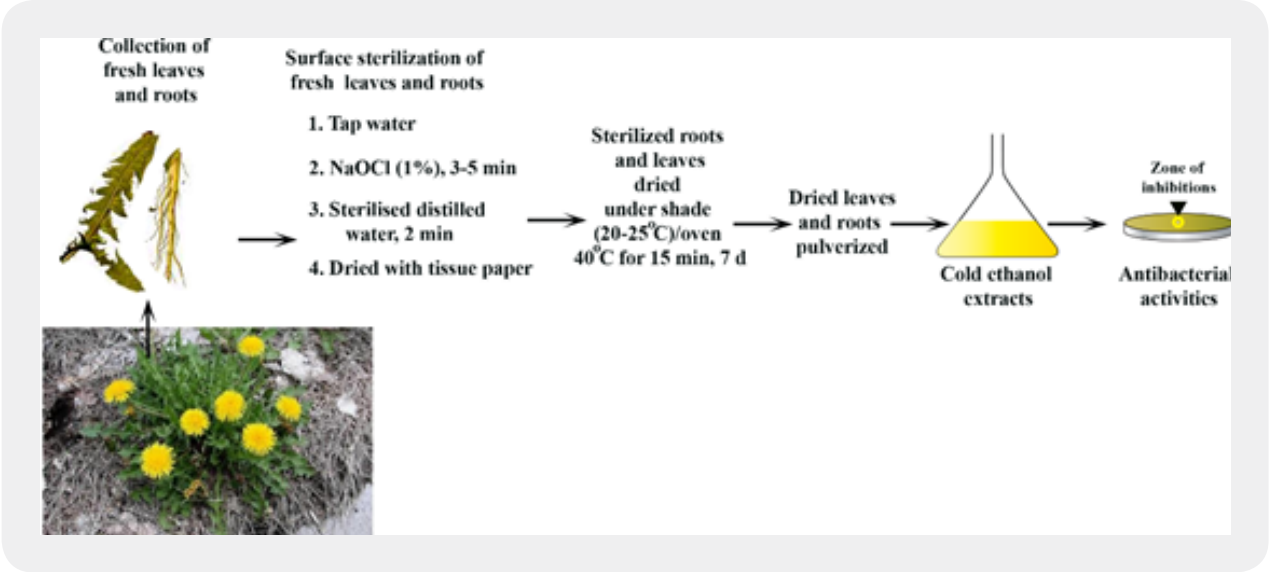

Figure 2: *Schematic representations of the experimental methodologies followed*

Antibacterial Activity Assay

The bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) and the pathogenic strain (*Streptococcus pneumonia*) were procured from the laboratory section of Tishreen University Hospital, Latakia, Syria and maintained on nutrient agar slants at 4°C. Each of the strains was streaked to a nutrient agar plate and incubated for 5 days at 37°C. A single pure colony of the respective bacterial strains was further inoculated into 5mL of Nutrient Broth (NB) and incubated at 37°C for 5 days. Following the incubation, the bacterial cultures were adjusted to a concentration of 10^6 cells/mL for the antimicrobial assay.

The ager disc diffusion method [39] was implemented to determine the antibacterial activities of the ethanolic *T. officinale* extracts. The grown bacterial cultures were inoculated at a concentration of 10^6cfu/mL in 20mL of molten agar media with a gentle shaking and poured in a Petri dishes (100mm x 15mm) and air-dried under laminar air flow (Esco Technologies, Pennsylvania, USA). The filter paper discs (6mm in diameter) were infused with 10µL ethanolic plant extracts, air dried and laid down on the agar media plate inoculated with the bacterial culture. The plates were incubated under aerobic conditions at 37°C for 48h. The antimicrobial activities were measured as the zone of clearance around each bacterial colony by subtracting the size of the infused disk from the zone of clearance observed.

However, the active components isolation and characterisation of the plant ethanolic extracts have not been performed in this study.

Results

Antibacterial Activity

The antibacterial activity of ethanolic extracts of leaves and root of *T. officinale* with the standard drugs

(TMP/SMX) showed from low to no inhibitory activities against all the tested bacterial strains (Table 1, Figure 3). The root extracts exhibited maximum inhibitory efficacy with zone of clearance measuring 24mm, 17mm and 12mm against *S. aureus, P. aeruginosa* and *E. coli* respectively while the leaves extract showed 14mm and 11mm against *P. aeruginosa* and *S. pneumonia* respectively. TMP/SMX (0.01mg/µL; positive control) showed strong antimicrobial effects against *E. coli* and *S. aureus* with zone of inhibition measuring 31mm and 30mm respectively while *S. pneumoniae* and *P. aeruginosa* showed no susceptibility towards the positive control, indicating that these microbes were found resistant to the drug. The negative control (water) does not exhibit any antimicrobial activities.

Table 1: *Antibacterial activity of ethanolic extract of T. officinale leavess and roots against bacterial strains. +/- : denotes Gram positive / negative, NZI: No zone of inhibition*

Sensitive bacterial isolates	Gram characteristics	**Ethanol extract (Zone of inhibition, mm)**		
		Leaves extract	Root extract	Trimethoprim/Sulfa-methoxazole
Staphylococcus aureus	+	NZI	24	30
Streptococcus pneumoniae	+	11	NZI	NZI
Escherichia coli	-	NZI	12	31
Pseudomonas aeruginosa	-	14	17	NZI

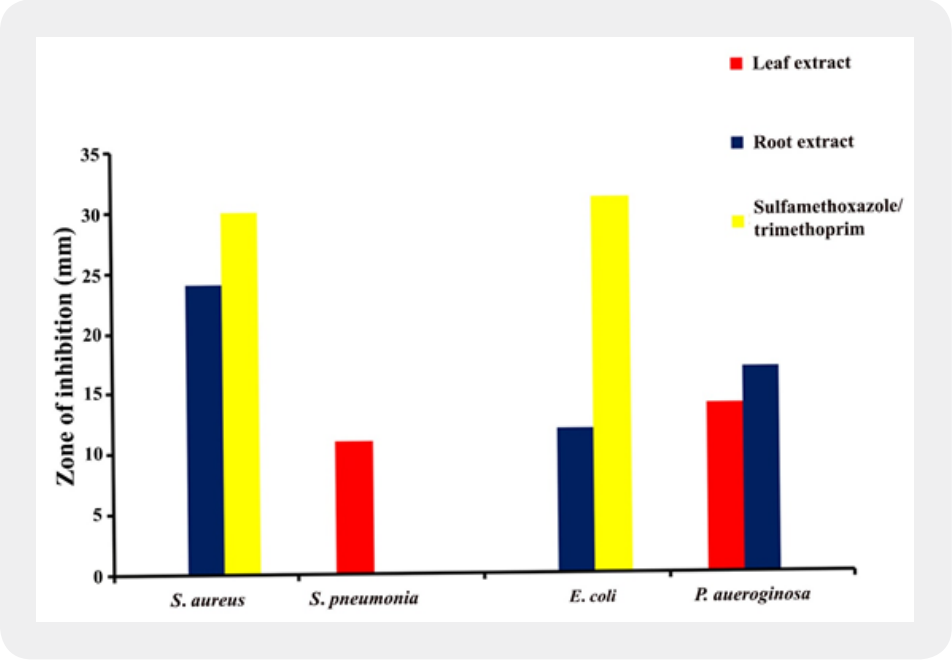

Figure 3: *Antimicrobial activities (zone of inhibition values) of ethanolic extract of T. officinale leaves and root, Trimethoprim/ sulfamethoxazole (0.01 mg/⌈L; positive control) tested against different bacteria strains.*

Discussion

In recent years, there has been a decline in microbial susceptibility to the existing antimicrobial agents liable for drug resistance in hospitals and in communities causing a global epidemic of antibiotic resistance, leading to an ecological disaster of unknown consequence [40,41]. Numerous studies [42-45] have divulged the importance for new antimicrobial agents to replace the arsenal of anti-infective agents. However, owing mostly to the financial glitches, a failure of antibiotic discovery remained unnoticed, and thus, no new classes of antimicrobial agent were expected to be in use in 20 years [40,42]. For this reason, the development and research into naturally derived compounds (polyphenolics and brassinosteroids) from plants and thus, their wide-range of biological properties and bioactive constituents were proven to be useful against many disease causative agents [46].

The current study explored the antibacterial activities of ethanol extracts of roots and leaves of *T. officinale* against subcultures strains of *S. aureus, S. pneumoniae, E. coli* and *P. aeruginosa. T. officinale* exhibited to have antibacterial activity for both the root and leaves extracts. In consistent to the present study, a previous study has also shown the antimicrobial activities of seven herbal extracts of *T. officinale* against the *Candida* strains [47]. Furthermore, another study has reported hydroethanol extracts of *T. officinale* showed about 26% inhibition against *H. pylori* [48]. The present study revealed that the root extracts showed stronger antagonistic activities in comparison to leaves extracts. However, the zones of inhibition exhibited by TMP/SMX against *S. aureus* and *E. coli* were more in comparison to the root extracts against the microbes. These findings were in accordance with earlier studies where the antimicrobial activities of *Taraxacum* extracts at 0.1mg/disc against *E. coli, P. Vulgaris, S. aureus*, and *B. subtilis* were relatively lower (zone of inhibition diameter =7.12 and 19.4mm) in comparison to gentamicin and tetracycline (18.9-38.8mm) [49,50].

Nonetheless, the root extracts gave a specific efficacy of 17mm inhibition diameter against *Pseudomonas aeruginosa* which is known for its antibiotic resistance and was not sensitive to the reference antibiotic (TMP/SMX). Also, root extract has an acceptable efficacy towards *E. coli*. Conversely, the root extract did not show any efficacy towards pulmonary bacteria *Streptococcus pneumoniae* in comparison to the leaves extract. The antibacterial activities of the root extracts against the antibiotic resistant strain could be attributed to the high content of phenolic compounds (secondary metabolites) in the roots in comparison to the leaves or other plant organs [17,28,51,52]. For example, a previous study tested the antimicrobial properties of crude and dialysed extracts from *T. officinale* root, where it has been further characterised by the presence of two hydroxyl fatty acids (NPF406) and three phenolic based compounds (NPF5) [28]. These compounds are likely to be responsible for the antimicrobial activity against *S. aureus* and *B. cereus*, thus, suggesting the use of dandelion root as a source of natural antimicrobial compounds. Furthermore, another study evaluated the total phenolic components (TPC) in *T. officinale*, where the highest phenolic contents obtained was in hydro-alcoholic extract (691.6mg/g GAE), thus, indicating significant role as antimicrobial agents [53].

Secondary metabolites from natural plant products are now the source for drug development. As they are produced in the living systems, it has shown more similarities to drugs and biologically compactible than synthetic drugs [54]. For instance, plant-derived drugs for anti-cancer are naturally derived compounds from plants, which are non-toxic to normal human cells, and thus, can be lead into clinical trials for further

therapeutic development [55]. However, plant medicines can also function as models for pharmacologically active compounds that may possess higher activity and less toxicity than their synthetic counterparts [56,57]. According to a previous study [58], the globally control of helminthic disease in humans using synthetic drugs are less effective, and also causing numerous side effects. Additionally, the proceeded employment of synthetic anthelmintic/larvicidal drugs show major resistant drug problem in diseases caused by parasites. Olive leaves extract is proven to cure upper respiratory illness such as nasal obstruction, sore throat, tonsillitis, and common cold [59]. The study reported that olive extract comprises of polyphenols, significantly oleuropein and hydroxytyrosol; having antiviral, antibacterial, anti-inflammatory and antioxidant properties which reduces the rate of these upper respiratory illness. Nonetheless, our study was the first where ethanolic *T. officinale* extracts were used to determine the antibacterial activity against bacterial strains in Syria. The importance of these results stem from the possibility of using the extracts of *T. officinale*, especially the root extract as a co-factor to antibiotics to eliminate multiple antibiotics resistant bacteria, especially *P. aeruginosa* and *S. aureus*.

Conclusions

Using diverse natural plant compounds as antimicrobial agents is an intriguing approach for discovering bioactive products used in alleviating sicknesses, in particular, cold, sore throat and flu. However, owing to the fact that plants are widely rich in variety of secondary metabolites, such as flavonoids, alkaloids, tannins and terpenoids, which have been found *in vitro* to have antimicrobial properties is important to plant medicinal research. Interestingly, our results for this study is the first documented report in Syria to the best of our knowledge. However, the results demonstrate that the ethanol root extract of *T. officinale* and TMP/SMX antibiotics is one of the drug that could be invested to take advantage of its antimicrobial compounds, especially as a co-factor for the treatment of bacterial infections resistant to antibiotics. This would assist to replace drugs to which bacteria have evolved resistance by encouraging traditional medicines. Moreover, further research on *T. officinale* by using different solvents may increase the efficacy rate of the plant product. Also, these findings can be helpful for the development of natural phytotherapeutic agents against diseases of humans and animals. The future prospects of this study should endorse to test other multidrug resistant bacterial strains such as Vancomycin-Resistant Enterococci and Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria. Furthermore, possibly it can also be extended to antiviral tests especially against virus such as sars2-cov-2 virus causing COVID-19. Additionally, the study can also be performed for cytotoxicity test against the animal cell lines (*in vitro*) and *in vivo* on model animals. Finally, this study will shed light on an important Mediterranean plant spread over in Syria and other parts of the Northern hemisphere in the hope that the research and studies will be completed in forthcoming projects.

Author Contributions

SG and RMH conceived the study and designed the experiment. RMH, MD, RA, RK and NK conducted all the experiments. SG and CCA drafted the manuscript. SG and RMH read and edited the manuscript.

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Conflict of Interests

The authors declare no financial interest or conflict of interest.

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