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Antibacterial and antioxidant potential of *Tetraena simplex* extracts of various polarities

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ABSTRACT

Nowadays, infectious and oxidative stress-related diseases are leading to many deaths worldwide. *Tetraena simplex*, a new species (synonym) that is mainly grown in Oman, has traditionally been used as a medicine for asthma. This study aimed to assess antioxidant and antibacterial activities of *T. simplex* extracts of various polarities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and agar gel diffusion assays. Among the six extracts prepared, ethyl acetate extract showed the highest antioxidant activity and hexane extract showed the lowest antioxidant activity. Antioxidant activity of the extracts decreased in the order of ethyl acetate > dichloromethane > water > butanol > methanol > hexane. Similarly, antibacterial activities, indicated as inhibition zones, of the six extracts at four concentrations were assessed against two gram-positive bacteria (*Streptococcus pneumoniae* and *Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and Proteus bacilli). No extract showed antibacterial activity against the tested bacteria at any concentration. Therefore, the ethyl acetate extract of *T. simplex* may be used as an antioxidant or a food supplement as an alternative to synthetic drugs.

1. Introduction

Typically, the medicines used for human diseases are mostly derived from plant and animal sources, and almost all available indigenous plant species may be used as medicines to cure different illnesses. Plants as herbal remedies have been used to treat various diseases since ancient times [1]. Plants are one of the richest sources of natural antioxidants, which have been proven to exhibit remarkable antioxidant properties in laboratories [2]. All parts of a plant, including fruits, seeds, peel, roots, and leaves, can be used as sources of natural antioxidants due to their biological properties [3–5]. Approximately 25 % of prescription drugs used to treat both curable and incurable diseases are derived from various plant and animal parts [3,5]. Traditionally, plants and their manufactured herbal products have been used to prevent or treat diseases [3]. Several studies have shown that consuming various vegetables or fruits as a part of our daily diet is beneficial for health and reduces the risk of developing diseases. Therefore, the risk of certain diseases is reduced due to antioxidants present in different plant and animal parts [6]. Antioxidants inhibit oxidative stress and subsequent cellular damage. As a result, such antioxidants have frequently been discussed for their health benefits and disease preventive potential. Some severe and chronic diseases such as diabetes, hypertension etc may be prevented through the consumption of fruits, seeds, peel, roots, and vegetables. Additionally, these substances remove oxidizing agents, which are potentially harmful to living organisms. The human body naturally produces free radicals to counter these harmful effects. However, in most cases, the levels of free radicals are much higher than those of naturally occurring antioxidants. There are various naturally occurring antioxidants, including beta-carotene, lycopene, proanthocyanidins, and flavonoids, which are effective in preventing diseases (Fig. 1).

Tetraena simplex (*T. simplex*) is a newly identified species of the family Zygophyllaceae. Over 53 species belonging to this family are known globally. Synonyms of *T. simplex* such as *Zygophyllum simplex* L or *Fabago portulacifolius* M are used in some Arabian countries. *T. simplex* has several common names such as arid, batbaak, aburukaiba, qarmal, harmal, and retreat. In Oman, it is known as harmal. *T. simplex* is a new species found in Gulf countries, including Oman [7]. It is an annual plant with red stems branching from the base. It is also native to North and Northeast Africa, Arabia, and east of India. The plant reaches the height of ~20–30 cm. The leaves are bright green or yellow and with entire margins, and the leaf arrangement is opposite. The flowers are bright yellow with five petals, and these are produced throughout the year. The fruit is an oval capsule with winged segments. Harmal is a halophyte growing in arid and semi-arid regions. Traditionally, *T. simplex* has been used in Arab states to treat asthma, gout, and swelling

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Fig. 1. Plant picture of Tetraena simplex.

[1,8]. This plant emits a foul odor, making it unattractive for animals to feed on. The plant contains phenolic compounds, non-alkaline mineral salts, alkaloids, flavonoids, cyclic diterpenes, amino acids, and glycosides, among other compounds [1,8,9]. Most plants belonging to Zygophyllaceae have traditionally been used to treat diseases such as cancer, non-malignant tumors, osteomyelitis, psoriasis, and warts [1,9,10]. Natural products derived from plants and animals are the main sources of new drugs, and these are the only choice worldwide at present. Microorganisms, animals, and plants are one of the most important natural sources of drugs. Among these sources, plants and their derived products are only safe and suitable owing to their renewability. Therefore, they play key roles in maintaining good health. Meanwhile, indigenous plants as sources of effective drugs and therapeutic agents have garnered much research attention in recent years. Such drugs are more effective than their synthetic counterparts and are often associated with negligible side effects [11]. In Oman, several plant species belonging to Zygophyllaceae are readily available and have traditionally been used to treat various illnesses such as cancer, diabetics, hypertension, and kidney and liver diseases [10]. In the United Arab Emirates and Oman, *T. simplex* has also been used to treat various eye infections and is often present in ophthalmic preparations. However, several studies suggest that there is no evidence of the biological, pharmacological, and chemical characteristics of *T. simplex* or of its alleged antimicrobial and antioxidant activities. Therefore, our aim was to prepare crude extracts of aerial parts of *T. simplex* with various polarities and to evaluate their antioxidant and antibacterial activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and agar gel diffusion assays.

2. Materials and methods

2.1. Reagents and chemicals

Several solvents such as hexane, ethyl acetate, butanol, methanol, dichloromethane (DCM), and acetone were obtained from Sigma Aldrich (Germany). Other essential reagents and chemicals such as Na_2SO_4 were obtained from BDH (UK). Levofloxacin, DPPH, and dimethyl sulfoxide (DMSO) were obtained from Fluka (Germany).

2.2. Sample collection

T. simplex samples were collected from Izz, Manah, Al-Dakhiliya, Oman, on Monday, September 17, 2018, at approximately 10.00 pm. The collected samples were stored in plastic bags to be transported to the laboratory for research. The morpholocal identification was done by Dr. Syed Abdullah Gilani, Associate Professor, College of Arts and Sciences and the identification number was deposited in the Research Lab. Aerial parts were used. Clean aerial parts were dried at room temperature for 1 week and ground into coarse powder. The powered samples were used for extraction.

2.3. Microorganism

Five pathogenic microorganisms were used: two gram-positive bacteria including *Streptococcus pneumoniae* (*S. pneumonia*) and *Staphylococcus aureus* (*S. aureus*), and three gram-negative bacteria including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and Proteus bacilli (P. bacilli). The strains were obtained from Nizwa Hospital at the end of December 2018. The collected bacterial strains were cultured in the biology laboratory at the College of Arts and Science of the University of Nizwa, Nizwa.

2.4. Extract preparation

Normal tap water was used to clean the samples at room temperature. The samples were dried in the shade for 5 days until totally dried. The dried samples were ground to powder using a kitchen blender. Total weight of the ground samples was 402.43 g. Subsequently, the ground samples were extracted with 1.5 L methanol using Soxhlet extraction for 45 h [3–6]. The solvent was slowly removed from the methanol extract using a rotary evaporator. Following this, 1 g residue was separated and stored in a vial. The remaining residue was mixed with 200 mL distilled water; this mixture was then transferred to a separatory funnel to be fractioned using different solvents with various polarities including hexane, DCM, water, ethyl acetate, methanol, and butanol. In total, 30 mL of each solvent mixture was added and shaken in the separatory funnel for 20–30 min; a researcher periodically checked and released the pressure from the separatory funnel [3–6]. The aqueous portion was evaporated in the same way to obtain the aqueous extract. Weight of each crude extract was recorded as follows: 0.7 g of hexane extract, 11.98 g of DCM extract, 0.58 g of ethyl acetate extract, 1.5 g of butanol extract, 1.0 g of methanol extract, and 10.6 g of aqueous extract.

2.5. Antioxidant potential

Antioxidant activities of crude *T. simplex* extracts with various polarities at different concentrations were estimated using a DPPH method. Antioxidant activity was tested using a modified DPPH assay [3–6,19–21]. The DPPH solution was prepared by dissolving 3.3 mg DPPH powder in 100 mL methanol to obtain a concentration of 2.7 mM. In this study, five concentrations of 200, 100, 50, 25, and 12.5 μg/mL were used for all the extracts. First, 200 μg/mL of each crude extract was prepared by dissolving 2 mg powder of each extract in 10 mL methanol in six different test tubes. Then, 100 μg/mL concentration was prepared by diluting the 200 μg/mL concentration by removing 5 mL from each test tube to another six tubes; concentrations of 50, 25, and 12.5 μg/mL were prepared in the same manner. We then transferred 300 μL of each concentration into different labeled test tubes and added 900 μL of methanol to each to make the volume 1.2 mL. The mixture was mixed well, and 300 μL of 2.7 mM DPPH was added. Thereafter, the test tubes were incubated in the dark for 1.5 h, and absorbance of each sample was measured using a UV spectrometer at 517 nm [3–6,12]. This test was repeated three times. Antioxidant activity of the crude extracts expressed as percent inhibition was calculated according to the following formula:

$$\% \, Inhibition = \frac{A\,(control) - A\,(extract)}{A\,(control)} \times 100$$

2.6. Antibacterial potential

Antibacterial activities of the six crude extracts prepared from aerial parts of *T. simplex* were determined using the agar disc diffusion assay against two gram-positive *S. pneumonia* and *S. aureus*, and three gram-negative bacteria *E. coli*, *K. pneumoniae*, and P. bacilli. A total of 10 mg of each crude extract was dissolved in 5 mL DMSO in different test tubes to obtain a concentration of 2000 μg/mL; this concentration was diluted to obtain concentrations of 1000, 500, and 250 μg/mL. The standard was prepared by dissolving 1 mg levofloxacin in 1 mL DMSO. A 5-mm disc was prepared using a filter paper. The bacterial strains were inoculated on different agar plates. The discs were soaked in different concentrations of the samples; DMSO was used as a negative control and levofloxacin as a positive control. The agar plates were incubated at 37 °C for 24 h. Finally, the inhibition zones were measured using a ruler and documented in mm [12–15]. This test was performed in triplicates.

3. Results

Several studies have shown that consuming vegetables or fruits as a part of our daily diet is beneficial for health and reduces the risk of developing diseases. The risk of certain diseases is reduced due to antioxidants present in different plant and animal parts [6]. Some severe and chronic diseases such as diabetes, hypertension etc may be prevented through the consumption of fruits, seeds, peel, roots, and vegetables. Additionally, these substances remove oxidizing agents, which are potentially harmful to living organisms. The human body naturally produces free radicals to counter these harmful effects. However, in most cases, the levels of free radicals are much higher than those of naturally occurring antioxidants. There are various naturally occurring antioxidants, including beta-carotene, lycopene, proanthocyanidins, and flavonoids, which are effective in preventing diseases.

3.1. Crude extract

The plant powder samples were extracted with various solvents by using Soxhlet method and the yield is presented in Table 1.

3.2. Antioxidant potential

Crude *T. simplex* extracts of various polarities were used to determine their antioxidant activities via a modified DPPH assay [11,19–21]. After 1.5 h incubation, the absorbance was measured and the percentage of inhibition (%) was calculated for each extract. Ethyl acetate extract showed the highest antioxidant activity, and hexane extract showed the lowest antioxidant activity (Table 2). Antioxidant activity of the extracts decreased in the order of ethyl acetate > DCM > water > butanol > methanol > hexane (Table 2).

3.3. Antibacterial potential

Inhibition zones of different crude *T. simplex* extracts against five pathogenic bacteria were tested using agar gel diffusion assay [3,18]. As a positive control, the broad-spectrum antibiotic levofloxacin was used; as a negative control, DMSO was used. After 24 h of incubation, diameters of inhibition zones were measured for each extract (Table 3). No crude extract showed antibacterial activity against the tested strains.

Table 1
Yield of each extract of *T. simplex*.

Extracts	Yield of extract (g)
Hexane	0.7
Dichloromethane	11.98
Ethyl acetate	0.58
Butanol	1.5
Water	10.6
Methanol	29.99

Table 2
Antioxidant activity of various polarity extract of *T. simplex*.

Extract	Conc. (μg/mL)	Absorbance (Wavelength 517 nm)	Percentage of inhibition %
	DPPH	0.811	-
	12.5	0.565	30.00 ± 0.21
Hexane	25	0.476	41.30 ± 0.17
	50	0.460	43.30 ± 0.54
	100	0.490	39.58 ± 0.97
	200	0.421	48.08 ± 0.11
	12.5	0.472	41.81 ± 0.34
Methanol	25	0.494	39.12 ± 0.24
	50	0.499	38.50 ± 0.72
	100	0.466	42.50 ± 0.22
	200	0.437	46.12 ± 0.45
	12.5	0.509	37.24 ± 0.91
	25	0.503	37.98 ± 0.10
Butanol	50	0.500	38.35 ± 0.77
	100	0.406	49.94 ± 0.23
	200	0.445	45.13 ± 0.55
	12.5	0.412	49.20 ± 0.39
	25	0.379	53.26 ± 0.18
Ethyl acetate	50	0.378	53.40 ± 0.12
	100	0.356	56.00 ± 0.54
	200	0.433	46.61 ± 0.76
	12.5	0.445	45.13 ± 0.20
Water	25	0.395	51.29 ± 0.09
	50	0.371	54.25 ± 0.23
	100	0.385	52.53 ± 0.27
	200	0.461	43.15 ± 0.19
	12.5	0.451	44.38 ± 0.40
DCM	25	0.425	47.59 ± 0.61
	50	0.429	47.10 ± 0.23
	100	0.401	50.55 ± 0.61
	200	0.347	57.20 ± 0.16

Each value is a mean of three biological replicates.

4. Discussion

Plants have been known for many centuries as a source of medicinal compounds. The World Health Organization has reported that nearly 80 % population in developing countries relies on plant-based medicines as a primary source of health care [5,16,17]. Plants provide an alternative treatment to local populations who cannot afford commercially available synthetic drugs. When the therapeutic effects of these plants are verified, they will gain wide acceptance by the scientific community. Natural compounds can also provide pharmaceutical industries with a foundation for the development of highly effective synthetic compounds. Currently, it is estimated that at least 121 prescription drugs are derived directly from plants [13]. Traditional healers have used plant preparations to treat many diseases, such as malaria.

Many studies have investigated medicinal uses of various plants, with a primary aim of discovering new natural drugs with less side effects. In fact, many studies have identified novel plants resources to discover new drugs for severe illnesses. Many researchers are trying to avoid the use of synthetic drugs by replacing these with natural ones. Sultanate of Oman is known for its great diversity of plant sources. In Oman, *T. simplex* plant was not traditionally used; however, in the United Arab Emirates, this plant was used for treating eye infections and in ophthalmic preparations. To date, no study has demonstrated antioxidant and antimicrobial activities of *T. simplex*, although some studies have reported such properties of other species of Zygophyllaceae. To this end, the aim of this study was to assess the antioxidant and antimicrobial activities of *T. simplex*. We prepared crude extracts of different aerial parts of *T. simplex* samples collected from Oman and evaluated their antioxidant and antimicrobial activities. *T. simplex* is a new species reported in certain regions of Sultanate of Oman. Traditionally, people in Oman have used different types of herbs and therapies to cure many illnesses. More recently, scientists have discovered new active compounds including antibiotics and

Table 3
Antimicrobial activity of various polarity extract of *T. simplex*.

Extract	Conc. (μg/mL)	*E. coli* (mm)	*K. pneumoniae* (mm)	*P.* bacilli (mm)	*S.pneumoniae* (mm)	*S. aureus* (mm)
	DMSO	nd	nd	nd	nd	nd
	2000	nd	nd	nd	nd	9 ± 0.10
Hexane	1000	nd	nd	nd	nd	nd
	500	nd	nd	nd	nd	nd
	250	nd	nd	nd	nd	7 ± 0.18
	Control	nd	50 ± 0.17	15 ± 0.16	15 ± 0.52	30 ± 0.55
	2000	nd	10 ± 0.11	nd	nd	10 ± 0.37
Methanol	1000	nd	nd	nd	nd	nd
	500	nd	8 ± 0.72	nd	nd	nd
	250	5 ± 0.22	25 ± 0.32	nd	nd	5 ± 0.10
	Control	nd	30 ± 0.33	10	nd	26 ± 0.12
	2000	nd	nd	nd	nd	nd
	1000	nd	nd	nd	nd	nd
Butanol	500	nd	nd	nd	nd	nd
	250	nd	nd	nd	nd	nd
	Control	9 ± 0.17	47 ± 0.09	15 ± 0.39	nd	23 ± 0.54
	2000	nd	10 ± 0.21	6 ± 0.14	10 ± 0.27	nd
	1000	nd	nd	nd	nd	nd
Ethyl acetate	500	nd	nd	nd	nd	nd
	250	nd	nd	9 ± 0.72	nd	nd
	Control	5 ± 0.17	38 ± 0.65	nd	nd	30 ± 0.51
	2000	nd	nd	nd	nd	nd
Water	1000	nd	nd	nd	nd	nd
	500	nd	6 ± 0.44	nd	nd	nd
	250	nd	8 ± 0.15	nd	nd	nd
	Control	nd	30 ± 0.11	15 ± 0.11	nd	27 ± 0.80
	2000	nd	nd	nd	nd	nd
DCM	1000	nd	nd	nd	nd	8 ± 0.32
	500	nd	nd	nd	nd	nd
	250	nd	nd	nd	nd	nd
	Control	nd	40 ± 0.43	10 ± 0.10	nd	32 ± 0.25

Control = Levofloxacin; nd = not detectable; DCM = Dichloromethane; DMSO = Dimethyl sulphoxide.

anticancer agents in Omani medicinal plants. *T. simplex* is not distributed worldwide, limiting its familiarity. It is reported from some parts in Africa, Arabia, and northern parts of India. Moreover, *T. simplex* is a medicinal plant that has locally been used for treating different diseases including eye infections. Furthermore, it has been used in the ophthalmic preparations. There are many rare plant species worldwide, including in Oman.

4.1. Antioxidant potential

The effective role of antioxidants is their interaction with oxidative free radicals. During the DPPH process, antioxidants respond with the steady free radicals of α,α-diphenyl-β-picrylhydrazyl (deep color) and gradually exchange or adapt them to produce DPPH derivatives, resulting in a color change [3,4,18]. No color indicates the antioxidant activity of the sample, such as for phenolic compounds, alkaloids, glycosides [8,9,19–21]. Our results show that the six aerial crude extracts of *T. simplex* neutralized the free radicals present in the solution. Generally, secondary metabolites in plants proved vital to humans because of their antioxidant contents. Differences in antioxidant concentrations were compared to gallic acid concentration, one of the major natural antioxidants in plants, widely used as a standard measurement for antioxidant values. The percentage of antioxidant capacity was measured to determine the scavenging activity, resulting in an increase of lipid peroxidation inhibitory molecules. Nevertheless, this value might include all types of antioxidants present in the sample, thus the variation in antioxidant properties was unable to be accurately measured. There are also plant extracts that have high DPPH radical scavenging actions. These variations are likely because of their diverse geographical distribution or sample collection and processing [15]. Therefore, the stronger in phenolic activities also take into account to specify smaller groups of active antioxidant compounds in *T. simplex*. The selected types of plants are already distributed only in some Arab countries and Oman is one of these. The plant is new and there are no research has been done on the selected plant species. In addition, this plant species was used traditionally by the Arabian ethnic communities to treat various ailments. Therefore, in this study, we selected this plant to screen the biological activity of the aerial parts with multipolar crude extracts. There are reports are available on antioxidant activity for other species belongs to this family, but not *T. simplex* [5–9]. However, our results are not supported by their results. The difference in results could be due to the extraction, preparation of crude extracts and the dose.

4.2. Antibaerial potential

The experimental antibacterial results obtained from this study are presented in Table 3. Almost none of the crude extracts from the aerial parts of *T. simplex* showed any antibacterial activity against the bacterial strains used [3,5,13,15], with the exception of *E. coli* in methanol extract. The aerial methanol crude extract at concentrations 250 μg/mL showed the minimum activity with the range of 5 mm against *E. coli* bacterial strain. Hexane and DCM did not show any activity at any concentrations against *K. pneumoniae*. However, methanol aerial extracts at all concentrations except 1000 μg/mL showed activity within the range of 0–25 mm. The lowest concentration methanol extract gave promising activity against *K. pneumoniae.* The ethyl acetate at concentration 2000 μg/mL and water at 500 and 250 μg/mL also gave activity within the range 0–10 mm against *K. pneumoniae. P.* bacilli did not show any activity against any of the polarities, except with ethyl acetate at 250 μg/mL and 2000 μg/mL within the range of 0–9 mm. On the other hand, *Streptococcus pneunoniae* did not show any activity. Almost all crude extracts at all concentrations except ethyl acetate 2000 μg/mL with the value of 10 mm. It is noticed that low concentrations of crude extracts have high inhibition zones. The ethyl acetate, butanol, and water extracts did not show any activity against *Staphylococcus* *aureus*. However, the hexane, methanol, and DCM extracts gave inhibition of the variety of 0–10 mm against *Staphylococcus aureus*. Our research findings illustrate that extracts are not sensitive to high concentrations. The antimicrobial activity of the plant's crude extracts relies on the bioactive phytochemicals e.g., alkaloids, flavonoids, saponins, glycosides, steroids, etc. [18]. The antibacterial data cannot be compared with the reported antibacterial data due to a lack of data on the antibacterial activity of *T. simplex*. Several reports are available on the antibacterial activity of other plants belonging to the Zygophyllaceae family [19,22,23]; however, our results are not aligned with their conclusions. The variation of results may result from the preparation of crude extracts, bacterial strains and dose applied.

5. Conclusion

In this study, we evaluated the antioxidant and antimicrobial properties of different crude extracts from the aerial parts of *T. simplex*. In addition, we observed no antibacterial activity in most extracts at almost every concentration. The high antioxidant activity, crude extracts from *T. simplex* can be used as a natural, safe antioxidant medication to treat different ailments or as food supplements as well. The chosen plant is an unusual and uncommon plant and it is newly detected in the Arabian countries exactly in the Gulf countries as well. Yet, there is little scientific evidence available on the biological and phytochemical properties of *T. simplex*. Consequently, additional *in vivo* and *in vitro* biological and pharmacological studies will be required to confirm its antibacterial and antioxidant properties. More research is also required to classify and separate the bioactive compounds in *T. simplex*, as well as their mechanisms of action.

CRediT authorship contribution statement

Ahmed Moussad Abdulsattar: Data curation, Investigation. **Mohammad Amzad Hossain:** Conceptualization, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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