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Original Article

Phytochemical Analysis and Antibacterial Studies of Some Yemeni Medicinal Plants against Selected Common Human Pathogenic Bacteria

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Abstract

In traditional medicine, medicinal plants are often used to treat various infectious diseases in humans. **Objective**: This study aimed to identify the phytochemicals contained in thirteen parts of six Yemeni medicinal plants: *Artemisia Judaica, Ficus carica L., Nerium oleander L., Olea europaea, Santolina chamaecyparissus L.* and *Withania somnifera* and to investigate the antimicrobial activity of different concentrations of methanol extracts from the aerial part of the selected plants against *S. saprophyticus*, *Salmonella* and *E. coli* in vitro. **Methods**: Phytochemical screening was carried out using standard procedures and the antibacterial activity of different extracts of each plant was studied by agar well diffusion method. **Results**: Phytochemical analysis of the plant extracts revealed the presence of phenols, saponins, essential oils, flavonoids, steroids, tannins, cardiac glycosides and alkaloids. The occurrence of phytochemicals varied from plant to plant. Tannins and phenols were found in all samples, while all other test samples contained at least five of the phytochemicals tested. Almost all extracts were able to inhibit the growth of the bacterial strains, with the exception of the species *S. saprophyticus*, which was resistant to the methanolic extracts of *A. judaica* and *F. carica L*. However, the methanol extract (0.1 g/ml) of *S. chamaecyparissus L.* and O. europaea showed the largest zone of inhibition (35 mm and 20 mm, respectively) against *S. saprophyticus*. This is the first report on the antibacterial activity of *S. chamaecyparissus L.* species against *S. saprophyticus*. **Conclusions**: The present screening result shows that the methanol extract of the tested traditional Yemeni medicinal plants exhibits potent antibacterial activity, and the studied plants may represent a new source for the discovery of novel antibacterial compounds for the treatment of drug-resistant human pathogens.

Keywords: Phytochemical Analysis; Agar Well Diffusion Assay; Antibacterial Activity.

1. Introduction

Since ancient times, natural remedies have been an important part of the treatment and prevention of diseases in humans. In primary health care, especially in developing countries, natural products made from medicinal plants are becoming increasingly important [1]. Although the Yemeni population frequently uses herbal treatments, only a few species of the local flora have been studied experimentally [2].

Ficus carica L. is an important member of the genus Ficus. Phytochemical studies on *F. carica* have revealed the presence of numerous bioactive compounds such as flavonoids, vitamins, nicotinic acid, tyrosine, sapogenin, ficusin, bergaptene, psoralen, taraxasterol, rutin and furanocoumarin [3, 4]. On the other hand, previous studies on crude extracts of *F. carica* have discovered a broad spectrum of biological activities. The most interesting therapeutic effects include anticancer, hepatoprotective, hypoglycemic and hypolipidemic effects [5-7]. *Artemisia Judaica L.* is widely distributed in the Mediterranean region, including Egypt, Saudi Arabia, Jordan, and Yemen. Previous phytochemical studies on *A. Judaica L.* have identified several classes of natural products, such as hispidolin, cirsilineol, oxygenated monoterpenes and sesquiterpenes [8]. The biological studies of the essential oils of *A. judaica* have shown various effects, including helminthic, anti-inflammatory, analgesic, antioxidant, antimicrobial and antipyretic properties [9-11]. *Nerium oleander* is the only listed species of the genus Nerium and is used in traditional medicine to treat various diseases. Previous pharmacological studies have shown that *N. oleander* possesses antioxidant, anticancer, antiparasitic, analgesic, dermatologic, cardiovascular, anti-inflammatory, hypolipidemic, and central nervous system effects [12,13]. The most important phytoconstituents of this plant include cis- and trans-carenin, neriumoside, kanersoid, oleandrin, folinrin, adenerin, nerin, cardenolides, bufadienolides and digitoxigenin [14]. *Olea europaea L.* is a small tree belonging to the Oleaceae family. It is native to tropical and warm temperate regions of the world. Phytochemical studies on Olea europaea have led to the isolation of triterpenes, flavonoids, flavonoid glycosides, iridoids, iridate glycosides, secoiridoid glycosides, biophenols, benzoic acid derivatives and other secondary metabolites [15, 16]. *Santolina chamaecyparissus L.,* also known as cotton lavender, is considered an aromatic plant. Several studies have described the essential oil composition of species growing in different Mediterranean regions and in India [17-19]. *Withania somnifera* is one of the most important species belonging to the genus Withania. Chemical investigation of various plant parts of *W. somnifera* has identified numerous compounds such as sitoindoside, anaferin, withanolides, withaferins and isopelletierin [20, 21]. The plant studied, family, part used, local name and traditional use are summarized in Table 1.

There are several reports on antibacterial activities from natural resources, focusing on antibacterial and/or antifungal activities associated with natural products against various diseases [22]. For the identification and development of potential new drugs against microbial diseases to reduce the development of resistance and adverse treatment effects, folk medicine offers a valuable and underutilized resource [23]. Scientific research therefore continues to prioritize the screening of plants for their therapeutic properties. It is well known that infectious diseases are the leading cause of death in the world, and the emergence of multidrug-resistant organisms threatens the clinical efficacy of many current drugs [24]. For this reason, ongoing efforts are being made to develop new antimicrobial agents, whether through their design and synthesis or through the discovery of natural sources of antimicrobial agents. Therefore, this study aimed to assess the phytochemical constituents and antimicrobial potential of methanolic extracts from the aerial parts of six Yemeni medicinal plants against three clinical pathogenic isolates.

2. Materials and Methods

2.1 Plant materials

Different parts of six Yemeni medicinal plants were collected in January 2021 from their natural habitat in Dhamar city, South Yemen for various areas (Table 1). They were identified by Mona Saleh Al-Sabari from the Faculty of Agriculture, Thamar University.

2.2 Preparation of Extracts for Antibacterial Activity

The collected plant material was first dried in the shade, ground and then 200 g of the dry powder of each aerial part of the plant material was soaked in methanol for 72 hrs at room temperature. The procedure was carried out three times. After the solvent was evaporated at 45 °C under reduced pressure in a vacuum rotary evaporator, the crude extracts were obtained. The resulting methanol extract of each plant was stored until use. The methanolic extracts were tested for their antibacterial activity.

2.3 Phytochemical Tests

Screening of the plant material for various phytochemical constituents was performed according to a standard procedure [37-39] as described in Table 2.

2.4 Antimicrobial activity assay:

2.4.1 Antibacterial activity of plants extract

The antimicrobial activity of the extracts was tested at various concentrations of 100-0.8 mg/ml. The methanol extracts of the tested plant samples were weighed and dissolved in DMSO to prepare a stock solution with a concentration of 100 mg/ml. The same stock solution was used to obtain the desired concentrations of 50, 25, 12.5, 6.3, 3.2, 1.6 and 0.8 mg/ml by the serial dilution method using the equation, $C_1V_1 = C_2V_2$, where C = concentrations and V = volume.

2.4.2 Microorganisms

Salmonella, *Escherichia coli*, and *Staphylococcus saprophyticus* were isolated from clinical samples of patients visiting the microbiology laboratory at Dr. Mohamed Khaled Hospital, in Dhamar City, Yemen. The organisms were isolated in nutrient agar medium and selectively cultured at 37 °C for 24 hrs. The bacterial strains were identified using standard biochemical tests.

2.4.3 Antibacterial activity Screening.

The antibacterial activity of the methanol extracts of the tested plant samples was determined using the agarwell diffusion method [40, 41]. The media used were Muller-Hinton agar. 0.1 ml of a freshly grown culture of the test organisms was spread evenly on the surface of the solidified agar using a sterile spatula. Wells with a diameter of 6 mm were made in the agar plate. Approximately 100 µl of different plant extracts were added to the wells and the plates were incubated at 37 °C for 24 hrs. Amoxicillin (30 µg/disk), doxycycline (30 µg/disk) and cefuroxime (30 µg/disk) were used as positive controls. The solvent control (DMSO) was included in each experiment as a negative control. Antibacterial activity was determined by measuring the diameter of the inhibition zones (mm).

Table 1: List of plants screened and their traditional uses.

Species	Family	Part used	Local name	Traditional uses	Reference
Artemisia Judaica L.	Asteraceae	Aerial part	Euthiran	Treatment of gastrointestinal disorders, enhanced eyesight, immune systems, capillary strength, arthritis, and cardiovascular health	[25-27]
Ficus carica L.	Moraceae	Leaves	Altiyn	Colic, indigestion, loss of appetite, diarrhea, sore throats, cough, bronchial problems, inflammatory, and cardiovascular disorders	[28, 29]
Nerium oleander L.	Apocynaceae	Leaves	Defla	Antidiabetic, abortifacient, itching, antigale, against hair loss, eczema and dental rages. used in snake and other venomous bites	[30-32]
Olea europaea L.	Oleaceae	Leaves and Stems	Zaytun	Antirheumatic, leishmaniasis, inflamed gums, diabetes, anti-gout, hypertension, and skin diseases of animals (camels)	[33, 34]
Santolina chamaecyparissus L.	Asteraceae	Aerial part	Qaisum	Analgesic, digestive, antispasmodic, fungicidal, bactericidal and antidiabetic	[18, 35]
Withania somnifera	Solanaceae	Leaves	U'beb	Ear pain, wound healing, and Burns	[36]

Table 2: Preliminary phytochemical tests for plant extracts.

Phytoconstituents	Test procedure	Observation
Alkaloids (Mayer's test)	2ml extract + 2 drops HCl + few drops of Mayer's reagent	White precipitate
Tannins (Braymer's Test)	2ml extract +2ml H_2O +2-3 drops $FeCl_3$ (3%)	Green precipitate
Saponins (Froth test)	5ml extract + 5ml H_2O + heat	Froth appears
Flavonoid (Shinoda test)	2ml extract + a piece of Mg ribbon + 3 drops conc. HCl	Reddish coloration
Phenols (Ferric chloride test)	2 ml extract + 4 drops $FeCl_3$ dilute	Blue-black coloration
Steroids (Liebermann-Burchard test)	2ml extract + 3 drops $(CH_3CO)_2O$ + few drops H_2SO_4	Brown ring at the junction
Cardiac glycosides (Killer Killiani test)	2ml extract + 1ml CH_3COOH +3 drops $FeCl_3$ + 2 drops H_2SO_4	Reddish brown at the junction
Volatile oils	2ml extract + 2ml HCl (1%)	White precipitate

3. Results and Discussion

3.1. Phytochemical profiling

Preliminary phytochemical analysis is the simplest method for the detection of secondary metabolites in plant extracts. Phytochemical constituents such as tannins, saponins, alkaloids, flavonoids, glycosides and several other aromatic compounds are secondary metabolites of plants that serve as a defense mechanism against attack by many microorganisms, insects and other herbivores. The result of phytochemical screening of the extracts showed the presence of different bioactive compounds in most of the selected plants (Table 3), which could be responsible for the observed antibacterial property. Tannins and phenols are present in all parts of the selected plants. Tannins are generally used as a remedy for inflammation, hemorrhoids, burns, gonorrhea and in tanning [42]. Saponins were found in 5 plant samples, with saponins mainly present in the leaves and fruits of *W. somnfera*. It was found that plants containing large amounts of saponins have an antibacterial effect [43]. Essential oils are present in all plants studied, except in the bark of the stems of *S. chamaecyparissus* and *W. somnfera* and in the fruits of *F. carica L*. Essential oils have been shown to be effective in the treatment and prevention of a variety of diseases, including antiseptic, carminative, antifungal, antiviral and asthmatic treatment [44]. Cardiac glycosides have been found at low levels in the fruits of *N. oleander* and the stem bark of *S. chamaecyparissus*. Cardiac glycosides are used to treat microbiological infections, constipation, edema and congestive heart failure [45]. Flavonoids are present in all tested plants except *O. europaea*. The flavonoids are responsible for their positive antioxidant properties. Alkaloids are found in four plant samples, with alkaloids occurring mainly in the flowers of *N. oleander*, the fruits of *W. somnfera* and the leaves of *O. europaea*. Alkaloids have been reported to have narcotic, antispasmodic, antibacterial, antimalarial and analgesic properties [46, 47].

3.2 Antibacterial activity

The antibacterial effect of methanolic extracts of six Yemeni medicinal plants has been studied against pathogenic bacteria, especially *Salmonella*, which causes diarrhea, fever and stomach pain. *E. coli* is the most common bacterium whose virulent strains can cause gastroenteritis, urinary tract infections and meningitis in newborns, and *S. saprophyticus*, which causes urinary tract infections. It is also responsible for complications such as acute pyelonephritis, epididymitis, prostatitis and urethritis. The results of testing the antibacterial methanolic extract on the test strains are shown in Table 4. Of the plant extracts tested, the methanolic extract of *S. chamaecyparissus* showed the highest activity of 35 mm (0.1 g/ml) and 30 mm (0.0125 g/ml) zone of inhibition against *S. saprophyticus*, followed by the methanol extract of *O. europaea* with 20 mm (0.1 g/ml) zone of inhibition against *S. saprophyticus.*

However, the results of this study show that the methanolic crude extract of *S. chamaecyparissus* contains components such as tannins and flavonoids with significant antibacterial properties, which enable the extract to overcome the cell barrier of Gram-negative bacteria [48]. This is the first report of antibacterial activity of this species against S. saprophyticus. Most of the studies revealed that the essential oil of *S. chamaecyparissus* showed the highest antibacterial activity against different bacterial strains [18, 49, 50]. On the other hand, S. saprophyticus was resistant to the different concentrations of methanol plant extracts of *A. judaica* and *F. carica L.*

Methanol extracts of *N. oleander L.* inhibited the growth of all bacteria tested, with the highest activity against *S. saprophyticus* with an inhibition zone of 18 mm observed at a concentration of 6.3 mg/ml. In a previous study [51], the petroleum ether extract from the leaves of *N. oleander* was tested against four bacteria (*E. coli, K. Pneumoniae, B. Subtilis* and *S. lutea*). The results showed that it was highly effective against the *E. coli* strain, with an inhibition zone of 1.9 cm at a concentration of 5.12 g/ml.

Table 3: Qualitative analysis phytochemicals in the selected medicinal plant

Medicinal Plants	*A. Judaica*	*F. carica*		*N. oleander*		*O. europaea*		*S. chamaecyparissus*			*W. somnfera*		
Part tested.	**Aerial parts**	**Fr.**	**L.**	**F.**	**L.**	**L.**	**S.**	**F.**	**L.**	**S.**	**Fr.**	**L.**	**S.**
Phenols	+++	+	++	+++	++	++	++	+	++	++	+	++	++
Saponins	+	-	+	-	-	+	+	+	-	-	+++	+++	-
Volatile oils	++	-	++	+++	++	++	+	+	++	_	+	++	-
Flavonoid	++	+	-	+++	-	-	-	+	++	+	+	-	-
Steroids	++	-	-	+	++	+	-	+++	++	+	-	+	-
Tannins	+	+	+	+	+	++	+	+	+	+	+	+	+
Cardiac glycosides	-	+	-	++	+	+	-	-	+	++	-	-	-
Alkaloids	-	-	++	+++	+	+++	+	-	-	-	+++	+	-

F = Flowers; L= Leaves; Fr = Fruits; S = Stem bark; + = present; − = absent; ++ = moderately present; +++ = highly present of phytochemical constituents.

Table 4: Antibacterial activity of different plant methanol extracts at various concentrations.

	***A. Judaica* (mg/ml)**								***F. carica L* (mg/ml)**							
Bacterial species	***100***	***50***	***25***	***12.5***	***6.3***	***3.2***	***1.6***	***0.8***	***100***	**50**	**25**	**12.5**	**6.3**	**3.2**	**1.6**	**0.8**
S. saprophyticus	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Salmonella	R	R	11	R	12	11	10	13	R	R	R	R	9	12	10	10
E. coli	R	R	R	R	14	12	10	9	R	R	R	11	R	R	11	12
	***N. oleander L* (mg/ml)**								***O. europaea* (mg/ml)**							
Bacterial species	***100***	***50***	***25***	***12.5***	***6.3***	***3.2***	***1.6***	***0.8***	***100***	***50***	***25***	***12.5***	***6.3***	***3.2***	***1.6***	***0.8***
S. saprophyticus	12	11	12	12	18	16	15	15	20	14	15	10	15	18	17	19
Salmonella	7	14	13	15	11	8	7	R	13	12	8	12	11	12	13	12
E. coli	8	11	15	13	11	7	11	R	12	12	13	12	12	11	11	R
	***S. chamaecyparissus L.* (mg/ml)**								***W. somnifera* (mg/ml)**							
Bacterial species	***100***	***50***	***25***	***12.5***	***6.3***	***3.2***	***1.6***	***0.8***	***100***	***50***	***25***	***12.5***	***6.3***	***3.2***	***1.6***	***0.8***
S. saprophyticus	35	19	19	30	18	15	12	R	18	16	15	13	R	R	R	R
Salmonella	8	10	13	13	13	14	14	14	R	R	11	11	11	13	11	12
E. coli	11	10	R	R	R	R	11	R	R	R	11	11	12	15	13	11

	Amoxicillin	**Cefuroxime**	**Doxycycline**
Bacterial species	***30 μg***	***30 μg***	***30 μg***
S. saprophyticus	15	R	12
Salmonella	13	11	13
E. coli	R	R	9

R = no zone of inhibition.

The bacterium most sensitive to the methanol extracts of *W. somnifera* was *S. saprophyticus* with an inhibition zone of 18 mm (0.1 g/ml). In contrast, the same extract resisted *Salmonella* and *E. coli* at the same concentration of 0.1 g/ml. According to previous studies, *W. somnifera* methanol extracts had good efficacy against *E. coli* with an inhibition zone of 23 mm at 0.1 g/ml [52].

The study showed a non-monotonic correlation between the concentration of the plant extract and bacterial inhibition, with lower concentrations showing greater efficacy than higher concentrations. This phenomenon is attributed to several factors. First, at lower concentrations, the bioactive compounds effectively bind to the bacterial cell surface receptors, inhibiting important functions and preventing bacterial growth. However, as the concentrations increases, the effectiveness of the excess bioactive compounds decreases due to saturation of the receptors, resulting in a reduced inhibitory effect. Antagonistic interactions among bioactive compounds within plant extracts also contribute. These interactions are less pronounced at lower concentrations, which favors synergistic inhibition. Conversely, these interactions increase at higher concentrations, reducing the overall inhibitory effect. Another aspect is the chemical degradation of bioactive compounds, especially in the presence of oxygen or light. Higher concentrations can accelerate degradation and reduce the availability and effectiveness of the compounds against bacteria. In addition, certain plant extracts induce bacterial resistance at higher concentrations, which triggers a stress response that neutralizes or degrades the bioactive compounds and weakens the overall inhibitory effect. The factors contributing to this non-monotonic relationship depend on the particular plant extract, the bacterial species and the experimental conditions [53-58].

4. Conclusions

In summary, the phytochemical analysis of six Yemeni plants confirms that these plants were rich in tannins, phenols, essential oils, saponins, flavonoids, alkaloids and cardiac glycosides. These phytochemicals make the therapeutic properties of the plants studied more potent. The results also showed antimicrobial activities against *S. saprophyticus, Salmonella* and *E. coli* strains. Based on the results, it is evident that the methanol extract of the tested plants has strong antibacterial activity. The tested plants could serve as a new source for the discovery of new antibacterial chemicals for the treatment of drug-resistant human infections.

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