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Research Article

Ethnobotanical, Phytochemical, and Biological Study of *Tamarix aphylla* and *Aerva javanica* Medicinal Plants Growing in the Asir Region, Saudi Arabia

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Abstract

In this study, *Tamarix aphylla* and *Aerva javanica*, plants used in folkloric medicine in the Asir region of Saudi Arabia were studied ethnobotanically, chemically, and biologically to assess their medicinal uses, phytochemical constituents, and biological activities. Ethnobotanical data were collected using semistructured interviews and the use values were calculated. A total of 61 informants were interviewed and results indicated high-value uses (0.9 and 0.7) for the plants. Phytochemical investigation indicated that the plants contained most of the phytochemicals that were tested for. The antioxidant activity was examined by assaying total phenolic content, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and hydrogen peroxide (H_2O_2) radical scavenging activity. .The bark extract of *T. aphylla* showed the best antioxidant effect in total phenolic content, DPPH, and H_2O_2 assays (278.02 ± 0.16 mg GAE/100 g; The half-maximal inhibitory concentration (IC_{50}): DPPH, 18.39 ± 0.62 μg/mL; H_2O_2, 252.94 ± 1.86 μg/mL), followed by extract of the *A. javanica* aerial parts (228.60 ± 2.09 mg GAE/100 g; IC_{50}: DPPH, 28.54 ± 0.53 μg/mL; H_2O_2, 154.17 ± 0.78 μg/mL) compared with ascorbic acid (DPPH: 27.27 ± 0.11 μg/mL; H_2O_2: 164.9 ± 0.37 μg/mL). The disc diffusion method performed for antimicrobial activity revealed weak activities in *T. aphylla* and *A. javanica* extracts (100 μg/mL) against six human pathogens. It was concluded that these plants possess therapeutic potential in the treatment of 18 types of ailments, which are important phytochemical constituents and antioxidant activities that justify their therapeutic uses in traditional medicine. Thus, this study laid sufficient background for further pharmacological research on extracts of these plants.

Keywords

ethnobotany, *Tamarix aphylla*, *Aerva javanica*, phytochemicals, total phenolic content, antioxidant, Asir region

Introduction

Ethnomedicinal documentation, combined with phytochemical and bioactivity screening, is a convincing method for identifying new drugs from medicinal plants. Medicinal plants are used worldwide in traditional medicinal practices. In the Kingdom of Saudi Arabia, medicinal plants still play an essential role in human health (Sher & Aldosari, 2012), and their uses in the treatment of diseases are still available among the tribal, local people, and medicinal healers (*Hakim* and *Atar*). The Kingdom of Saudi Arabia is one of the most affluent biodiversity areas in the Arabian Peninsula (Yusuf, Al-Oqail, Al-Sheddi, Al-Rehaily, & Rahman, 2014). In total, 2,250 species in approximately 142 families have been recorded in the flora of the Kingdom of Saudi Arabia (Seraj, Jrais, & Ayyad, 2014). Among these species, 309 genera containing 471 species in 89 families are used in ethnomedicine (Aati, El-Gamal, Shaheen, & Kayser, 2019).

Consequently, these species need to be evaluated for their medicinal uses, phytochemical constituents, and

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biological activities. Most of Saudi Arabia's flora species are found in the Asir region, located in the southwestern part of the country, which provides a precious resource of medicinal plants consisting of numerous medicinal herbs, shrubs, and trees (Alqahtani, Alkholy, & Ferreira, 2014). However, most of these species remain unexplored, and only a few studies have been conducted to evaluate their biological activities and chemical constituents. The plants selected for this study have long been used by the inhabitants of the study area for the treatment of various diseases.

Tamarix aphylla (L.) Karst. (Figure 1) is a moderately sized tree, often multistemmed, that produces a spreading crown of many stout branches and long, drooping, jointed twigs. It can reach up to 10 to 12 m in height. The plant is native to northern and eastern Africa, the middle-east, and parts of southwestern Asia (Hayes, Walker, & Powell, 2009). It is particularly prevalent along inland waterways and widely cultivated as a garden ornamental, windbreaker, and street tree (Marwat et al., 2008; Orabi, Taniguchi, Terabayashi, & Hatano, 2011). It is widespread, natural, and well grown in the Asir region in the plains and valleys (wadis).

Aerva javanica (Burm. f.) Juss. ex Schult. (Figure 2), an erect, branched perennial herb, has a wide distribution in all habitats in Africa and some of the Asian countries and is extensively scattered in various regions of the world (Judd, Campbell, Kellog, Stevens, & Donoghue, 2008). It is commonly distributed in the southwestern parts of Saudi Arabia (Alwadie, 2005), particularly in the Asir region, where it has been widely used for its therapeutic effects in traditional medicine (Abulafatih, 1987).

These medicinal plants have already been studied elsewhere for their chemical composition and biological activities. However, the published data are scant regarding the ethnobotany, phytochemistry, and pharmacological properties of these Saudi Arabian species from the Asir region. Accordingly, our main objectives of this study were (i) to document the ethnobotanical

Figure 1. Photographs of *T. aphylla* (L.) Karst. (Saudi species): (a) Habit: whole tree, (b) branches with leaves, and (c) dried bark.

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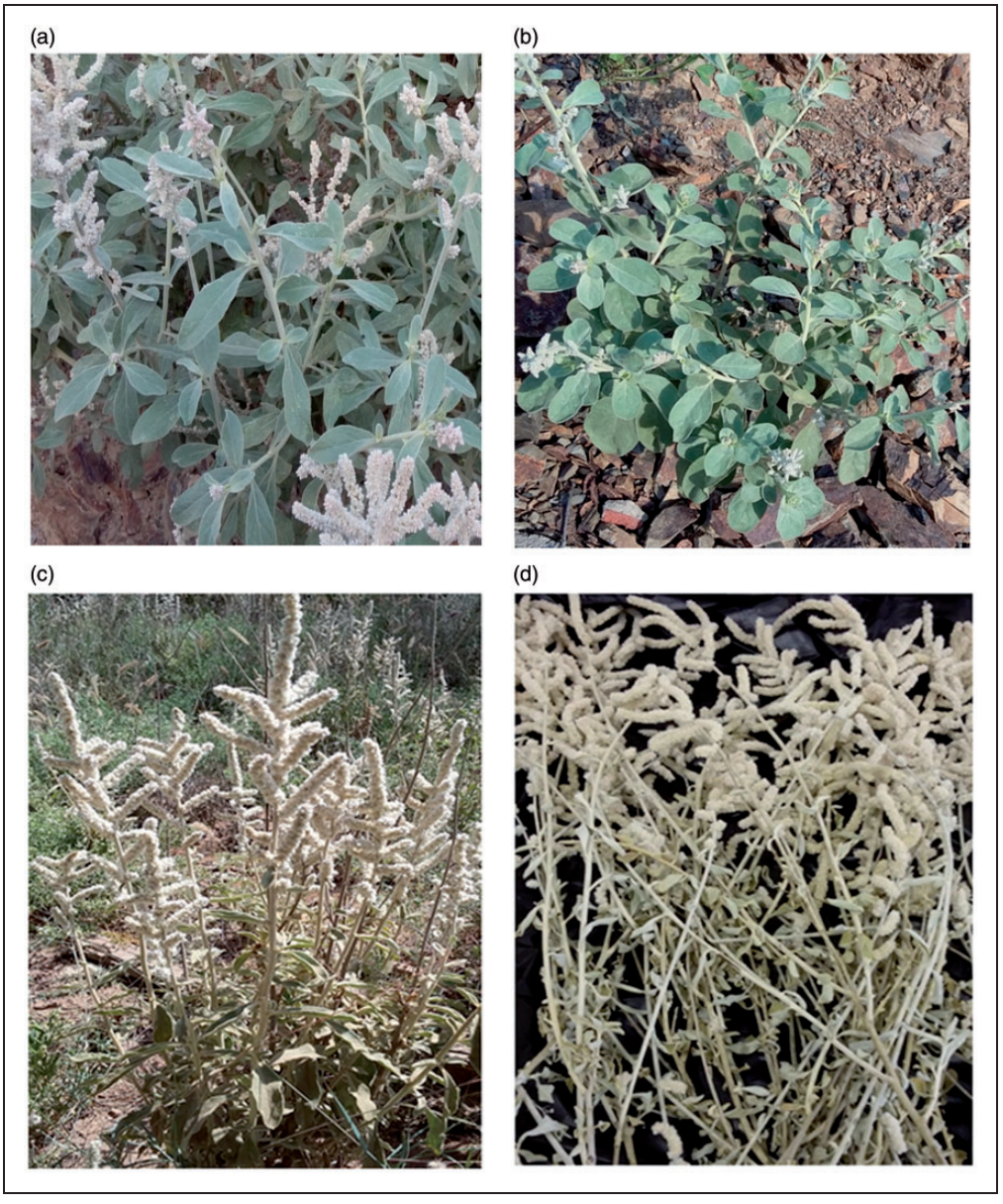

Figure 2. Photographs of *A. javanica* (Burm. f.) Juss. ex Schult. (Saudi species): (a to c) Habit: whole plant, leaves, and flowers and (d) dried aerial parts or branches with flowers.

knowledge related to these plants; (ii) to evaluate their phytochemical constituents, antimicrobial and antioxidant activities, and, consequently; (iii) to highlight the importance of these species, which would be beneficial for their conservation and facilitate future clinical studies on them.

Methods

Study Area

The study area, the Asir region (Figure 3), is located in the southwestern part of Saudi Arabia between latitudes 17°25′ and 19° 50′ North and longitude 50°00′ and 41°50′ East (El-Juhany & Aref, 2013). The region is situated on a high plateau of an area approximately 81,000 km^2, with the elevation ranging from 1,000 m to 3,130 m above the sea level. It contains mountains, slopes, rolling lands, rocky hills, deep valleys, and wadis. It is characterized by high rates of rainfall and moderate temperatures throughout the year (Seraj et al., 2014). The textures of the mountain soils of the region range from loamy silt to silty clay to sandy loam with a high percentage of organic matter, reflecting the favorable climatic environment for plant development. Such

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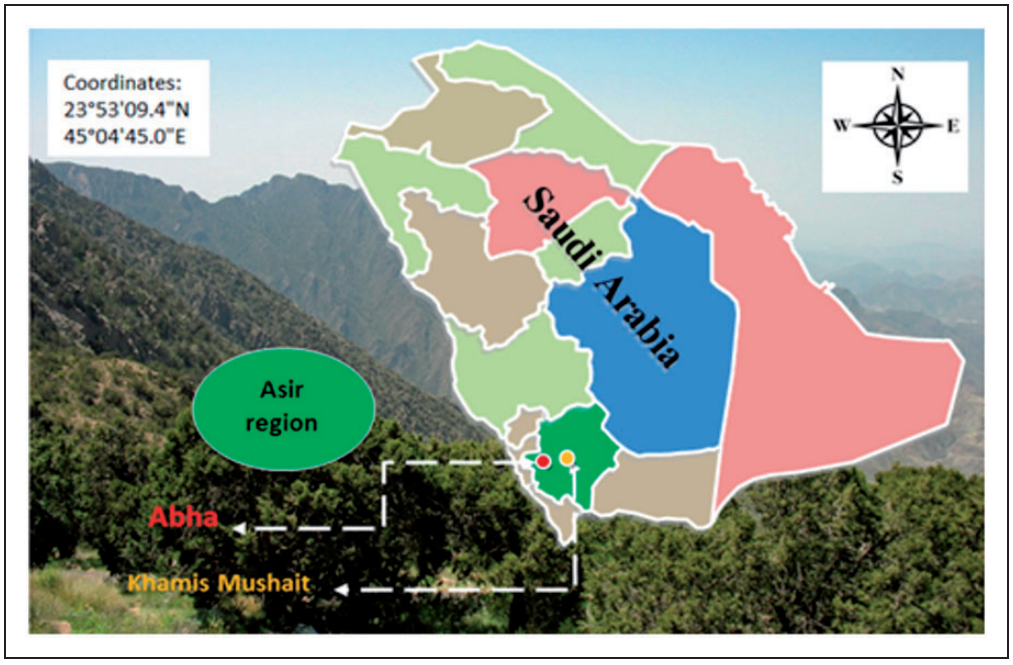

Figure 3. Map of the Saudi Arabia showing its 13 regions and the Asir region (in green) with its two main cities where the survey was carried out. Abha is located at 18° 13′ 0.4692″ N *latitude* and 42° 30′ 13.5540″ E *longitude*s, and it is situated at an elevation of 2,228 m above the sea level. Khamis Mushait is located at 18° 19′ 45.7824″ N *latitude* and 42° 45′ 33.7140″ E *longitude*s, and it is situated at an elevation of 1,998 m above the sea level. This image is prepared with the help of an editable map of Saudi Arabia (Adapted from https://yourfreetemplates.com).

topography, along with a relatively mild climate, creates a great diversity of plant communities and habitats for various plants species (Al-Qahtani, 2000; Aref, 1996), such as *Acacia* species, *Juniper* trees, *Ziziphus spina Christi*, *T. aphylla*, *Dobera glabra*, *Adenium obesum*, *Mimusops laurifolia*, *Ficus sycomorus*, *Tamarindus indica,* and many others herbs and shrubs (Masood & Asiry, 2012). The population of the region is estimated to be 2,212 million (The General Authority for Statistics, 2017). The capital of Asir region is Abha. In addition, the region contains some main cities, including Khamis Mushait, Bisha and Bareg, and their surrounding suburbs.

Botanical Identification

Several ethnobotanical field trips were carried out in the area around Abha and Khamis mushait cities during September/December 2018 to collect voucher specimens and photograph the selected plants. The plant's species were authenticated using The Plant List (2013) and the available relevant scientific publications of Saudi Arabia Flora (Chaudhary, 1999, 2001). Further confirmation was made with the assistance of Dr. E. Warag, Professor, Department of Biology, King Khalid University, Saudi Arabia. Voucher specimens were processed using standard taxonomic procedures (Bridson & Foreman, 1998) and were deposited for future reference at the Herbarium of Biology Department, College of Science, King Khalid University.

Ethnobotanical Data Collection and Literature Review

Ethnobotanical information on the selected plants was obtained using semistructured interviews with local people (older age), traditional medicine practitioners (*Hakim*), and local herbal drug sellers (*Atar*) who live or work in Abha and Khamis Mushait cities. Study participants were asked (in Arabic) about their knowledge of the three selected plants using the local name of the plant and the plant images. Specifically, other local names (if any) of each species, the medicinal uses, the diseases commonly treated with the plant, the plant part (s) used, and the modes of preparation were recorded.

A systematic literature review using different databases such as Google search, Google Scholar, and the Saudi Digital Library was conducted to collect and assess the documented information about the selected plants with a focus on the Saudi species. The literature review surveyed all the available documents and covered medicinal uses, biological and pharmacological activities, and chemical constituents of the selected plant species using the search terms "*Tamarix aphylla*," "*Aerva javanica*," "Medicinal Plant," "Traditional Medicine," "Ethnobotany," "Saudi Arabia," and "Asir region" independently or in combination.

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Use Value

The use value (UV) index (Andrade-Cetto & Heinrich, 2011) was used to evaluate the relative importance of the plant species based on its relative use among informants, according to Equation 1:

$$UVi = \frac{\Sigma Ui}{ns} \quad (1)$$

where *Ui* is the sum of the total number of use reports by all informants for a given species *i*, and *ns* is the total number of informants. A high UV indicates that the plant is important, and a low UV suggests few reports are related to its use.

Plant Samples and Extract Preparation

After confirming plant identity with informants, the plant samples including the bark of *T. aphylla* and roots and aerial parts of *A. javanica* were collected from three locations in the study area. The plant materials were cleaned, air dried at room temperature, and powdered with a mechanical grinder. Each plant sample (250 g) was macerated in ethanol (80% v/v) at room temperature with occasional stirring. Each extraction was repeated 3 times, and the collected filtrates were combined and concentrated under reduced pressure using a rotary evaporator (RV 10, IKA®-Werke GmbH & Co. KG, Staufen, Germany). The obtained dried extracts were weighed to determine the percentage yield and then were stored in a refrigerator (4°C) until they were used for analyses.

Phytochemical Screening

Qualitative phytochemical screening was subjected to each plant sample extract to detect the different classes of phytochemicals present by adopting standard protocols (Harborne, 1998; Trease & Evans, 1989). The results were qualitatively expressed as the relative abundance.

Estimation of the Total Phenolic Content

The total phenolic content of plant extracts was estimated using the Folin-Ciocalteu spectrophotometric method as previously described by Nabavi, Ebrahimzadeh, Nabavi, Hamidinia, and Bekhradnia (2008), with minor modifications. The absorbance was measured at 760 nm using a JASCO V-530 UV/Vis spectrophotometer. Gallic acid (Sigma-Aldrich) was used as a standard to prepare the calibration curve. The total phenolic value in all samples was calculated and expressed as milligrams of gallic acid equivalents per 100 g of dry weight of the plant extract (mg GAE/100 g).

Estimation of the Total Flavonoid Content

The total flavonoid content of different plant extracts was estimated spectrophotometrically by the aluminum chloride assay described by Ordoñez, Gomez, Vattuone, and lsla (2006), with slight modifications. The absorbance of the treated sample and standard quercetin (Sigma-Aldrich) solutions were measured at 415 nm. Quercetin standards were used to produce the standard curve. The total flavonoid values in the test samples were calculated, and the results were expressed as milligrams of quercetin equivalents per 100 g of the dry weight of plant extracts (mg QE/100 g).

Evaluation of Antioxidant Activity

The antioxidant activity of the plant extracts was estimated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay as previously described by Eshwarappa, Iyer, Subbaramaiah, Richard, and Dhanajaya (2014) with minor modifications. The absorbance of the plant extract and standard solutions of ascorbic acid (positive control) were recorded at 517 nm. The percentage of inhibition of DPPH free radicals was calculated using Equation 2:

$$Inhibition\ (\%) = \frac{(Ac - As)}{Ac} \times 100 \quad (2)$$

where *Ac* is the absorbance of the control and *As* is the absorbance in the presence of the extract or standard. The half-maximal inhibitory concentration (IC_{50}), concentration of sample (in μg/mL) required to scavenge 50% of DPPH free radicals, was calculated by interpolation from the graph obtained by plotting the extract concentration (μg plant extract/mL of DPPH solution) versus percentage of inhibition.

The hydrogen peroxide (H_2O_2) scavenging assay, as reported by Ruch, Cheng, and Klaunig (1989), was also applied to estimate of the antioxidant activity of the plant extracts. The absorbance of the reaction mixture was recorded at 230 nm, and the H_2O_2 scavenging effects of both the extract and standard were calculated as percentages using Equation 2. The scavenging percentages calculated for the different concentrations of plant extracts were used to calculate the IC_{50} values using regression analysis.

Evaluation of Antimicrobial Activity

The plant extracts were screened for their activities against some human pathogens: Gram-positive (*Candida albicans* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae,* and *Shigella flexneri*) obtained from the Biology Department, King Khalid University.

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The pathogens were inoculated onto the nutrient agar plate and were incubated at 30°C for 48 h. The test was performed by the disc diffusion method (Berghe & Vlietinck, 1991; Ruangpan & Tendencia, 2004). The antimicrobial activity was calculated as the average zone (in mm) of pathogen growth inhibition. Ampicillin and nystatin (100 µg/mL) were used as positive controls for antibacterial and antifungal activity, respectively.

Statistical Analysis

Experiments were carried out in triplicate, and the data for each sample were recorded as means ± standard deviation ($n = 3$). MS Excel and SPSS (version 21) were used to perform statistical analysis. For all the analyses, the differences were considered to be significant at $p \leq .05$.

Results

Medicinal Values of the Plants

Table 1 represents the medicinal uses mentioned by the informants for each species. For each cited folk species, the scientific name, family, voucher number, local folk name(s), growth habit, part/medicinal uses, and UV were reported. Sixty-one selected informants, including 50 older men (50 to 72 years), 4 traditional medicine practitioners (*Hakim*), and 7 herbal drug sellers (*Atar*), were interviewed. The respondents confirmed more than one local name for each species. They also indicated that the best time to harvest the plants from the wild is in the spring before or during flowering. The ethnobotanical survey results revealed that the two plants could treat 18 types of diseases and disorders. Almost all the respondents knew about these species and confirmed that they were commonly used by the local communities to treat various human ailments. Most of the respondents reported that they had used these plants at least 1 time and that they advise neighbors and friends to use the plants. The typical plant parts mentioned by the study participants included leaves, bark, root, and aerial parts, while the preparation methods used included decoction, infusion, maceration, juice, powder, and paste. The most frequently reported ailments were rheumatism, wounds, skin diseases, pain, and kidney problems. The results showed that the same part was used to treat different conditions. For example, the leaves of *T. aphylla* were used to treat fever, wounds, jaundice, and rheumatism, and the roots of *A. javanica* were used to treat toothache, headache, kidney stones, and diarrhea. Generally, the UVs for the three plants were high (Table 1). *A. javanica* was the most frequently used species in the local area according to its high UV (0.9). It was mentioned by 90% of all the informants.

Table 1. List of Indigenous Selected Plants With Their Families, Local Names, Growth Habit, and Traditional Uses Recorded in the Study Area With Their UVs.

Scientific name/Family	Voucher number	Local name(s)	Growth habit	Part used and medicinal use(s)	UV
Tamarix aphylla (L.) Karst. Tamaricaceae	KH-1901	Athal Tarfaa	Tree	**Leaves:** - Decoction used for fever, tetanus, jaundice, and rheumatism. - Rubbed externally on the head to relieve headache and on legs as antirheumatic. - Smoke for wound healing. **Bark:** - Decoction to treat hepatitis. - Powder applied for eczema and other skin diseases. - As a poultice on wounds. **Roots:** - Paste applied directly on the wound to treat infections. - Decoction for stomach ache.	0.7
Aerva javanica (Burm. f.) Juss. ex Schult. Amaranthaceae	KH-1902	Arwa El Rowa Alreen Tuwaim	herb	**Aerial parts:** - Decoction used as a diuretic. - Maceration in water used to treat headache and hypertension. - Infusion or paste used to alleviate rheumatism. **Roots:** - Decoction used to relieve kidney stones. - Chewed for toothache and to relieve headache. - Paste is applied on the face to remove acne. - Infusion (drink) for diarrhea and chronic chest pain.	0.9

Note. UV = use value.

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Table 2. Documented Medicinal Uses, Biological Activities, and Phytochemicals of the Study Species.

Scientific name	Medicinal uses	Biological activities	Phytochemicals
Tamarix aphylla	**Leaves:** -Used to relieve headache and fever (Abulafatih, 1987; Said, Khalil, Fulder & Azaizeh, 2002). -Used for rheumatism (Ahmad, Zafar, & Sultana, 2009; Marwat et al., 2008). -Used for Wound healing, abscesses, jaundice, measles treatment, and as a pain killer (Marwat et al., 2008; Shaheen, Qureshi, Akram, & Gulfraz, 2014). -Decoction used for spleen swelling, tetanus, and gynecological problems (Benhouhou, 2005). **Bark:** -Used for hepatitis, skin diseases and syphilis (Akhlaq & Mohammed, 2011; Nawwar et al., 2009). **Root:** -Used for tuberculosis, smallpox, leprosy, and contagious ailments (Ali et al., 2019).	Antioxidant, analgesic and antipyretic activities (Al-Jaber & Allehaib, 2017; Mahfoudhi, Ben Salem, et al., 2016; Qadir, Abbas, Hamayun, & Ali, 2014). Antidiabetic (Mahfoudhi, Grosso, et al., 2016). Antimicrobial (Adnan et al., 2015; Alrumman, 2016; Bibi et al., 2015) Antileishmanial (Iqbal et al., 2012). Anti-inflammatory, antipyretic and analgesic (Al-Jaber & Allehaib, 2017; Iqbal et al., 2017; Yusufoglu & Alqasoumi, 2011).	Phenolic acids, flavonoids, alkaloids, triterpenes saponins, glycosides, and tannins (Adnan et al., 2015; Iqbal et al., 2012; Mahfoudhi, Grosso, et al., 2016; Nawwar et al., 2009; Umbetova, Choudhary, Sultanova, Burasheva, & Abilov, 2006). Gallic, caffeic, p-coumaric, ferulic and ellagic acids, kaempferol, quercetin, quercetin 3-O-galactoside, tamarixetin 3,30 -di-sodium sulfate, and dehydrodigallic acid (Mahfoudhi et al., 2016; Nawwar et al., 2009).
Aerva javanica	**Flowers:** -Decoction used to alleviate kidney problems (Deshmukh et al., 2008). -Paste used externally to heal wounds, to stop bleeding, and for inflammation of joints (Abulafatih, 1987; Samejo, Memon, Bhanger, & Khan, 2012). **Whole plant or aerial parts:** -Decoction used for chest pain, diarrhea, as a diuretic and demulcent, as a gargle to cure gum swelling and toothache (Arbab et al., 2016). -Decoction used for dysentery, gonorrhea, hyperglycemia, and cutaneous infections (Garg, Bhushan, & Kapoor, 1980; Khan et al., 2012). -Used as diabetic, diuretic, demulcent, and for skin diseases (Samejo et al., 2012). **Seeds:** -Decoction used for swelling, toothache, and headache (Arbab et al., 2016; El-Ghazali, Al-Khalifa, Saleem, & Abdallah, 2010; El-Shabasy, 2017). -Paste for constipation, boils, and pimples (Shaheen et al., 2014). **Roots:** -Paste to remove acne from the face (Perry & Metzger, 1980). -Used for kidney problems and rheumatism (Samejo et al., 2012).	Antiulcer (Khan et al., 2012). Hepatoprotective and nephroprotective (Arbab et al., 2016; Movaliya & Zaveri, 2014). Antidiabetic (Srinivas & Reddy, 2009). Antioxidant (Arbab et al., 2016; Singh, Jain, & Jain, 2010). Antiviral (Baltina et al., 2003). Antiplasmodic (El-Hadi, Barki, Yousif & Hassan, 2010; Simonsen et al., 2001). Antimicrobial (Khader, Ahmad, Abd Elsalam, Ullah & Islam, 2012; Mufti et al., 2012; Sharif, Ahmed, Hussain, Malik, & Ashraf, 2011).	Triterpenes, sterols, glycosides, alkaloids, flavonoids, saponins, tannins, and carbohydrates (Arbab et al., 2016; Nawaz, Shad, Andleeb, & Rehman, 2015). 3-hydroxy-4 methoxybenzaldehyde, ursolic acid and (E)-N-(4-hydroxy-3-methoxyphenethyl)-3-(4-hydroxy-3-ethoxyphenyl) acryl amide (Khan et al., 2012).

T. aphylla reflected a UV of 0.7. The literature search results are presented in Table 2 with the relevant references. The results revealed that different species of *T. aphylla* and *A. javanica* from other countries were previously studied for chemical constituents and biological and pharmacological activities. Various phytochemicals, such as tannins, alkaloids, saponins, glycosides, and flavonoids, were recorded for each of the two species along with different isolated phytochemical compounds.

Extraction Yield and Phytochemical Profiling

The extraction yields of ethanol extracts of the bark of *T. aphylla* and roots and aerial parts of *A. javanica* were 12.3%, 5%, and 6.7%, respectively. The results of preliminary phytochemical screening, represented in Figure 4, revealed the presence of alkaloids, glycosides, saponins, triterpenes, tannins, and flavonoids with different abundance levels in the three plants extracts. All the extracts tested negatively for anthraquinones.

Total Phenolic and Flavonoid Content and Antioxidant Activity

In this study, the total phenolic content and total flavonoid content of *T. aphylla* and *A. javanica* extracts were calculated using the linear equations obtained from gallic acid ($y = 9.6851x + 0.0353$, $R^2 = .9993$) and quercetin ($y = 31.904x + 0.0272$, $R^2 = .9996$) standard curves, respectively. Their antioxidant activities were also determined at different concentrations using DPPH and H_2O_2 radical scavenging assays with ascorbic acid used as a standard antioxidant. The IC_{50}, a parameter widely used to measure the antioxidant efficiency, was calculated from the graph obtained by plotting the inhibition (%) against concentrations values and was expressed in microgram per milliliter of the extract. The results are summarized in Table 3, where a lower IC_{50} value indicates a higher antioxidant activity. The results revealed that *T. aphylla* bark extract had the highest content of phenolic compounds (278.02 ± 0.16 mg GAE/100 g) followed by aerial parts extract of *A. javanica* (228.60 ± 2.09). The highest total flavonoids content was recorded in aerial parts extract of *A. javanica* (99.24 ± 0.07 mg QE/100 g). The antioxidant results showed potential free radical scavenging activity in a concentration-dependent manner. Based on the DPPH assay, the antioxidant capacity of the extracts calculated as the IC_{50} value in μg/mL was found to be 18.39 ± 0.62 and 28.54 ± 0.53 for *T. aphylla* (bark) and *A. javanica* (aerial parts), respectively, compared with the standard antioxidant ascorbic acid (27.27 ± 0.11). However, for the H_2O_2 assay, the highest antioxidant activity ($IC_{50} = 154.17 \pm 0.78$) was exhibited by *A. javanica* aerial parts extract followed by *T. aphylla* bark extract ($IC_{50} = 252.94 \pm 1.86$) comparing with ascorbic acid ($IC_{50} = 164.90 \pm 0.37$).

Antimicrobial Screening

Each plant extract, at the concentration of 100 μg/mL, was screened for antimicrobial activities. The results, graphically represented in Figure 5, showed that *T. aphylla* bark extract and *A. javanica* root extract exhibit moderate to weak activity against all the six tested human pathogens compared with the standard antibiotics (ampicillin and nystatin). However, the aerial parts extract of *A. javanica* showed no activity against the pathogens tested.

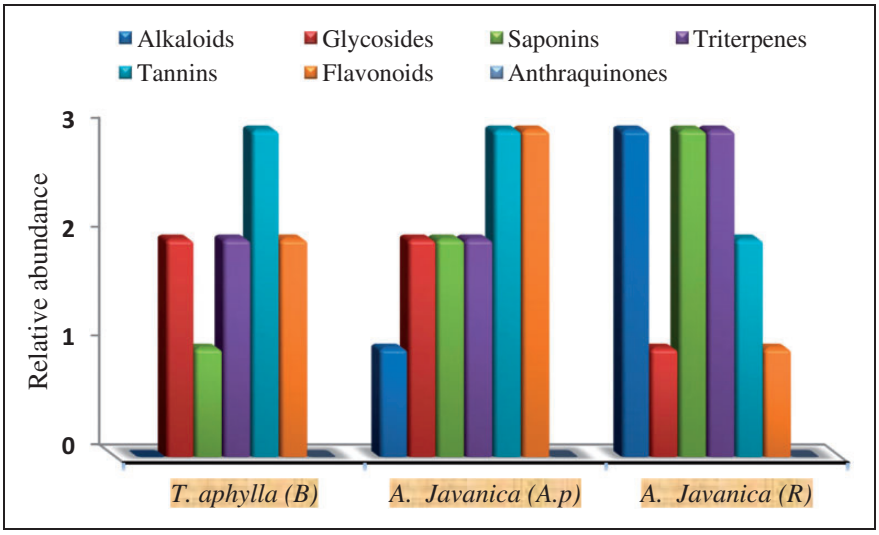

Figure 4. Phytochemical profile of the ethanolic extracts of the selected plants. **3** = abundant (heavy precipitate); **2** = fairly present (turbidity); **1** = slightly present; and **0** = absent (clear).

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Table 3. Total Phenolic Content (mg GAE/100 g Extract), Total Flavonoid Content (mg QE/100g Extract) and Antioxidant Activities of the Ethanol Extracts of *Tamarix aphylla* and *Aerva javanica* by DPPH and H_2O_2 Methods With IC_{50} (μg/mL) Values.

Plant species/ standard	TPC (mg GAE/100 g extract)	TFC (mg QE/100 g extract)	DPPH radical scavenging activity (% inhibition)	IC_{50} value (μg/mL)	H_2O_2 radical scavenging activity (% inhibition)	IC_{50} value (μg/mL)
T. aphylla (B)	278.02 ± 0.16	76.15 ± 2.01	45.28 ± 0.72 to 85.45 ± 0.88	18.39 ± 0.62	17.44 ± 0.99 to 78.76 ± 1.33	252.94 ± 1.86
A. javanica (A.p)	228.60 ± 2.09	99.24 ± 0.07	44.90 ± 1.32 to 72.42 ± 1.18	28.54 ± 0.53	20.68 ± 1.96 to 124.20 ± 2.82	154.17 ± 0.78
A. javanica (R)	122.46 ± 0.12	18.94 ± 1.66	32.50 ± 0.60 to 59.10 ± 0.44	272.54 ± 0.92	15.06 ± 1.20 to 54.76 ± 1.02	459.09 ± 2.39
Ascorbic acid	—	—	48.34 ± 0.68 to 76.50 ± 0.70	27.27 ± 0.11	11.94 ± 1.03 to 172.90 ± 2.01	164.90 ± 0.37

Note. Values are mean ± *SD* for triplicate experiments. TPC = total phenolic content; TFC = total flavonoid content; B = bark; A.p = aerial parts; R = root; DPPH = 1,1-Diphenyl-2-picrylhydrazyl; H_2O_2 = hydrogen peroxide.

Discussion

The UV index was used here to evaluate the relative importance of the plant species for the population in the study area. It is useful for the analysis of the use of a single species and in comparing plants among the same sample (Andrade-Cetto & Heinrich, 2011). Generally, the UVs for both plants were high, indicating that the informants have a great rate of dispersal of knowledge about the medicinal importance and uses of the plants studied. Plants with high UV reflected high usage by local people in the study area and great therapeutic effect against human health problems. Therefore, such plants should be focused on the investigation of bioactive phytochemicals and other pharmacological activities. The most frequently used species among the two plants is *A. javanica* with a 0.9 UV; most of the interviews mentioned *A. javanica* as the most commonly used and collected species. The ethnobotanical investigation has revealed that these two plant species provide remedies for approximately 18 human diseases in the study area that ranked them as high-value medicinal plant species. *A. javanica* aerial parts and roots are extensively used to treat various diseases, including rheumatism, headache, diarrhea, chronic chest pain, as a diuretic and to relieve kidney stones. Different parts of *T. aphylla* are used to treat many disorders, including joint pain, and skin and kidney problems.

Literature-Based Proof of Biological and Pharmacological Activities

Interestingly, some medicinal uses recorded in this study were consistent with those given in the literature, for example, the use of *A. javanica* as a diuretic (Arbab et al., 2016), and to remove acne from the face (Perry & Metzger, 1980), and the external use of *T. aphylla* for wound healing and skin diseases (Akhlaq & Mohammed, 2011; Marwat et al., 2008; Shaheen et al., 2014). Some medicinal uses were instead conflicting with the recorded applications, such as the use of *T. aphylla* root decoction for stomach ache that was previously cited for tuberculosis, smallpox, leprosy, and contagious ailments (Ali et al., 2019). In addition, *A. javanica* aerial parts extract was used to treat headache and hypertension; however, in the literature, it was used to heal dysentery, gonorrhea, hyperglycemia, and cutaneous infections (Garg et al., 1980; Khan et al., 2012). The present findings concerning the medicinal use of *T. aphylla* parts in wound treatment have been examined previously (Adnan et al., 2015; Iqbal et al., 2017). Yusufoglu and Alqasoumi (2011), who evaluated the wound healing activity of *T. aphylla* extract using the excision wound model to monitor wound contraction and wound closure time, reported that the plant extract

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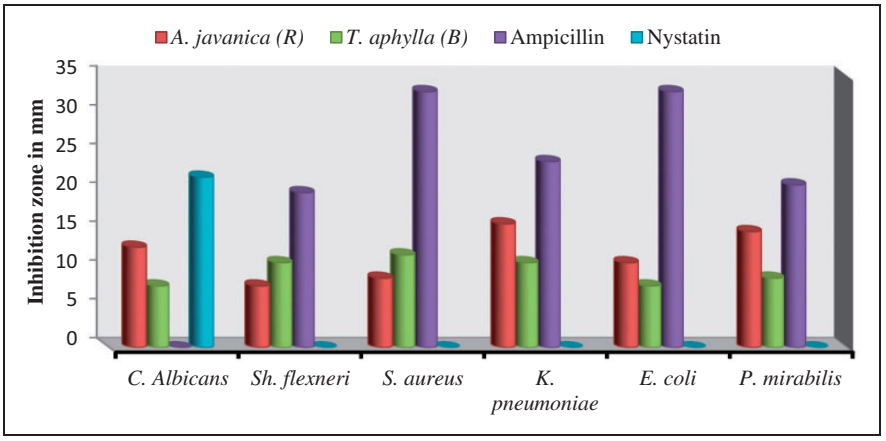

Figure 5. Graphical representation of the susceptibility study (expressed as a zone of inhibition) of the extracts of *A. javanica* root (R) and *T. aphylla* bark (B) against test pathogens.

accelerated the wound healing process by reducing wound contraction and wound half closure time. The use of the bark of *T. aphylla* to treat hepatitis, an inflammation of the liver caused by viruses, was also reported previously (Akhlaq & Mohammed, 2011; Nawwar et al., 2009) but was not confirmed in in vivo studies; hence, the plant extract has shown antioxidant activity (Mahfoudhi, Ben Salem, et al., 2016; Qadir et al., 2014) and a significant anti-inflammatory effect when tested in Wister albino rats using a carrageenan-induced paw edema test (Al-Jaber & Allehaib, 2017; Iqbal et al., 2017). A significant proportion of the respondents reported the use of *T. aphylla* leaves to relieve joint pain, headache, and rheumatism. The effectiveness of *T. aphylla* extract as an analgesic was assessed previously by Al-Jaber and Allehaib (2017) using two methods: acetic acid-induced writhing and the hot plate method in which both the alcoholic extract of *T. aphylla* showed high analgesic activity. Qadir et al. (2014) performed another earlier study using Eddy's hot plate method and demonstrated significant analgesic activities of *T. aphylla*. The phytochemical profile of *T. aphylla* bark extract, presented in Figure 4, indicated high amounts of tannins with moderate amounts of flavonoids, glycosides, and triterpenes, and the absence of alkaloids and anthraquinones. This result agrees with that recorded by Mahfoudhi, Prencipe, Mighri, and Pellati (2014) and Nawwar et al. (2009). Many active phytochemical compounds have been isolated and characterized from *T. aphylla* extracts (Table 2). The ethnobotanical results (Table 1) indicated that all parts of *A. javanica* have broad applications in folk medicine to treat various diseases. Aerial parts decoction of *A. javanica* is used to treat hypertension and headache and is used as a diuretic. This is possible, because diuretics are medications designed to increase the amount of water and salt expelled from the body as urine (Ellis, 2019) and are often prescribed to help treat high blood pressure, of which headache is symptom, but this use is not yet proven. Documented pharmacological activities of different parts of *A. javanica*, presented in Table 2, indicated the numerous biological activities of the plant. The phytochemical profile of *A. javanica* extracts is represented in Figure 4 and is comparable to the findings reported previously by many authors (Arbab et al., 2016; Nawaz et al., 2015). In addition, its root extract exhibited high amounts of alkaloids and saponins, whereas the aerial parts extract possessed triterpenes and flavonoids in abundance. The presence of such phytochemical constituents in these plants supports their biological activities and verifies their uses in traditional medicine to treat various diseases.

Antimicrobial Activities of the Extracts

From the present preliminary investigation, the root extract of *A. javanica* was found to display weak to moderate activity against all six tested pathogens (Figure 5). Similar results of the antimicrobial activity of *A. javanica* were reported previously (Mufti et al., 2012; Sharif et al., 2011). *T. aphylla* bark extract also showed weak to moderate activity against all the pathogens tested (Figure 5), and it was consistent with the findings obtained by Mahfoudhi, Ben Salem, et al. (2016). The weak antimicrobial activity of *A. javanica* and *T. aphylla* extracts suggested that the medicinal uses of these species may be attributing to biological properties other than antimicrobial properties.

Total Phenolic and Flavonoid Content and Antioxidant activity

Polyphenols are the most common and widely distributed group of secondary plant metabolites that are believed to assist in the prevention of oxidative stress

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and inflammatory diseases (Działo et al., 2016). The present results indicate that all the extracts tested contain a significant amount of phenolic and flavonoid content (Table 3), in agreement with the phytochemical profiles of these plants (Figure 4). The results also showed that *T. aphylla* and *A. javanica* extracts exhibited potential free radical scavenging activity in both DPPH and H_2O_2 assays in a concentration-dependent manner (Table 3). This finding likely correlates with their high total phenolic and flavonoids content because several studies implied that the antioxidant activity of plant extracts is strongly related to the polyphenol content (Ahmed et al., 2015; Kim, Chun, Kim, Moon, & Lee, 2003). The radical scavenging effect of the *T. aphylla* bark extract, which had a greater quantity of total phenolic compounds (278.02 ± 0.16 mg GAE/100 g), was also determined to be stronger in DPPH ($IC_{50} = 18.39 \pm 0.62$ µg/mL) and H_2O_2 (252.94 ± 1.86 µg/mL) assays. The total phenolic content of *T. aphylla* obtained in this study was lower than that reported by Mahfoudhi, Grosso, et al. (2016), whereas its antioxidant capacity was comparable to the findings obtained by Yusufoglu and Alqasoumi (2011) who evaluated the antioxidant activity of the ethanolic leaf extract of *T. aphylla* collected from the Al-Kharj region of Saudi Arabia. The aerial parts extract of *A. javanica* depicted high contents of both total phenolic (228.60 ± 2.09 mg GAE/100 g) and total flavonoids (99.24 ± 0.07 mg QE/100 g), whereas its root extract depicted moderate contents at 122.46 ± 0.12 mg GAE/100 g and 18.94 ± 1.66 mg QE/100 g for total phenolic and flavonoids, respectively. These results were higher than the values reported by Nawaz et al. (2015), who found that different extracts of *A. javanica* species from Pakistan contained 0.11 ± 0.01 to 3.54 ± 0.36 g GAE/100 g of total phenolic and 0.041 ± 0.001 to 2.02 ± 0.15 g QE/100 g of total flavonoids. *A. javanica* aerial parts extract exhibited high antioxidant activity in both DPPH ($IC_{50} = 28.54 \pm 0.53$ µg/mL) and H_2O_2 ($IC_{50} = 154.17 \pm 0.78$ µg/mL) assays compared with the standard antioxidant ascorbic acid (DPPH: $IC_{50} = 27.27 \pm 0.11$ µg/mL; H_2O_2: $IC_{50} = 164.90 \pm 0.37$ µg/mL). The present results were consistent with the findings obtained by Munir and Sarfraz (2014), but it was more potent than that achieved by Eltayeb, Eltayeb, and Salhimi (2017) who reported IC_{50} values ranging from 46.5 ± 2.2 to 78.3 ± 3.6 µg/mL in DPPH. Accordingly, Arbab et al. (2016) reported that *A. javanica* extract had the highest antioxidant activity in the b-carotene-linoleic acid bleaching assay. However, *T. aphylla* extract showed the lowest IC_{50} value in DPPH compared with ascorbic acid, confirming their potent antioxidant activity. Figure 6 also indicates good antioxidant activity of *A. javanica* extracts in both DPPH and H_2O_2 scavenging assays (low IC_{50} values). It could be significantly correlated with its total phenolic and flavonoid contents (Table 3). Owing to its good antioxidant activity, *A. javanica* has been reported to be used to treat chronic and degenerative ailments that come from oxidative damage caused by free radicals (Nawaz et al., 2015). These findings of antioxidant activities enhanced the capacity of *T. aphylla* and *A. javanica* extracts in the reported traditional medicinal uses and suggest that these plants may be considered potential sources of new antioxidant drugs.

Implications for Conservation

The ethnobotanical information concerning medicinal plants and their health benefits is still lying unclaimed in the Asir region of Saudi Arabia. This study has documented essential information regarding the ethnopharmacological and folklore uses of two plant species used by the inhabitants of the study area to treat approximately 18 human ailments. There was strong agreement among the informants in the uses of the plants.

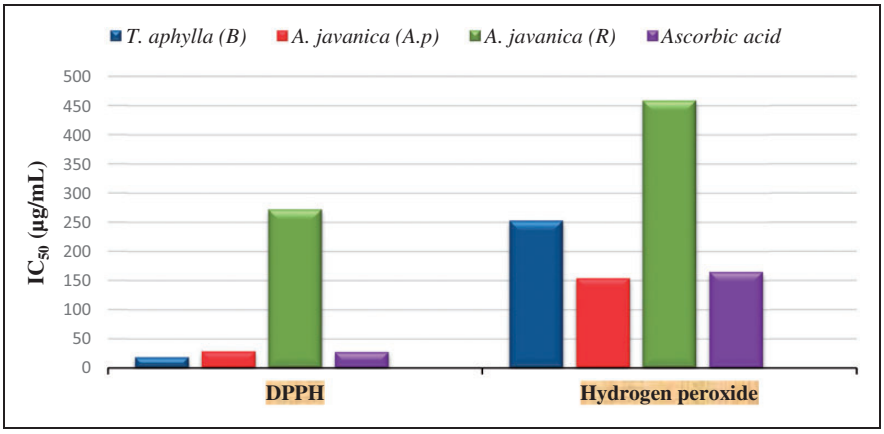

Figure 6. Comparison of the antioxidant capacities, IC_{50} (µg/mL) values of *T. aphylla* bark (B), *A. javanica* aerial parts (A.p), and *A. javanica* root (R), extracts and the standard antioxidant ascorbic acid in DPPH and H_2O_2 scavenging activities.

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The highest UV reported for the species indicated their importance in the traditional medicine of the area. The literature review showed that most of the therapeutic uses of these plants were similar to their medicinal uses in many other countries of the world. These findings highlight the significance of their traditional uses. The results revealed a significant yield for all classes of the biologically active phytochemicals. In addition, biological activity screening revealed a significant free radical scavenging activity. Hence, the traditional uses of these plants are likely to be due to their antioxidant potential and other unexplored biological properties. Thus, further research should be conducted on these medicinal plants to identify and isolate of compounds with antioxidant, anti-inflammatory, and analgesic activities for clinical use. This study has contributed to the documentation of indigenous knowledge on medicinal plants regarding the Asir region, one of the most affluent biodiversity areas in the Kingdom of Saudi Arabia. It has laid a significant background that can help to explore the medicinal values and create a database of medicinal plants available in Saudi Arabia, thereby contributing to the effort of conservation of these species.

Declaration of Conflicting Interests

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