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Assessment of Antimicrobial and Antioxidant Activity of methanolic extract from *Arnebia decumbens* aerial parts growing wild in Aja Mountain

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Abstract

Background: Many plants are a rich source of secondary metabolites with potential antimicrobial and antioxidant properties. Amongst the customary plant herbs used as a phytomedicine in Hail province, which has been selected for the current study, Kehail plant, *Arnebia decumbens* (*A. decumbens*). Therefore, this study sought to assess the antimicrobial and antioxidant activities against high-risk bacteria and yeasts.

Methods: The cup plate agar diffusion methods and Microdilution assays were adopted with minor modifications to assess the antibacterial activity. The Qualitative and quantitative tests were employed to assess the Kehail antioxidant activities by determining its phytochemicals, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ABTS(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and β-carotene *in vitro* assays.

Results: The results indicated that methanolic Kehail extract (M.K.E.) exhibited antibacterial activity against pathogenic bacteria, including *Staphylococcus epidermidis* (M13), *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Escherichia coli* ATCC 10536 and *Klebsiella pneumoniae*, while the mean inhibition zone was 10.66 ± 0.57 mm, 10.77 ± 0.57735 mm, 10.33 ± 0.57 mm, and 9.55 ± 0.57 mm, respectively. Furthermore, the antimicrobial activity increased in a concentration-dependent manner. Indeed, *E. cloacae* were the plant extract most inhibited bacteria. The plant extract has vigorous antifungal activity against *Candida albicans* ATCC 10231 and *C. tropicalis* ATCC 9362, whereas the mean inhibition zone was 12.77 ± 0.57 and 13.11 ± 1.52 mm, respectively. The extract of *A. decumbens* plant was also proven to be efficient as a source of antioxidants saponins, terpenes, polyphenols, and flavonoids.

Conclusion: The acquired outcomes uncover promising antioxidant activities of the tested Kehail methanolic extract. The study encourages the separation of active components and the development of new medications from the Kehail plant.

Introduction

The environment of the Hail region is rich in various annual and perennial plants. Its mountains and valleys are covered in the spring with green colors and rare types which may not be found in other regions. However, the natural vegetation in the region, especially the wild parks and area of Hail, which the people mean to go for a hike, is exposed to unjust logging. Despite the instructions and regulations, many plant species in the region are threatened with extinction [1], the trade and exploitation dependent on varieties, and the disappearance of Some plant species from the natural vegetation of the Hail region.

The mountainous regions' plants are fleshy; some species belong to the cactus family and the Euphorbiaceae family, especially their greasy species, dominates. The mountains' slopes develop into lush meadows with the onset of winter and the arrival of rain, especially in the mountains of Aja and Salma near the city of Hail [2]. The mountain plants are spread over large areas in these areas. These plants are utilized in medicinal concoctions that the ancients relied on to heal various disorders and their stunning beauty. The mountains of the Hail region contain about 140 species of mountain plants, both good and bad, including annuals and perennials, including the most well-known of these plants [3].

Arnebia species are rich in naphthoquinones such as alkannins, shikonins, and their subordinates, potent drug substances with many natural properties. Naphthoquinones are the significant phytochemicals in the external layer of Arnebia's species root. Naphthoquinones are made out of water-insoluble pigments such as shikonins, alkanes, and isohexenylnaphthazarin ester derivatives, which have pharmacological properties including anti-inflammatory, antimicrobial, injury-recuperating, and anti-tumorous activity [4]. Pyrrolizidine alkaloids, triterpene derivatives, flavonoids, and phenolic acids are other phytochemicals of Arnebia species [5, 6, 7].
Searching for new antimicrobial agents or a combination of drugs is necessary to combat new resistant pathogenic bacteria. It has been observed in previous studies synergistic effect of various plant extracts with antibiotics and drugs against some resistant bacteria [8].

Antioxidant function means capacity of a bioactive substance to keep up with cell design and capability by successful removal of free radicals, restraining peroxidation of lipids responses, as well as forestalling further oxidative damage [9]. It has been proposed that antioxidants could forestall numerous persistent sicknesses, such as cancer, diabetes, and cardiovascular disease [10]. This way, investigations, antioxidants found in nature, such as those found in medicinal plants, are significant. Plants produce a large cluster of helpful, pertinent, relevant secondary metabolites (phytochemicals) that display various therapeutic properties [11]. Most of these mixtures are utilized by plants to protect against different microorganisms, herbivores, and contenders [12]. Depending on their metabolic origins, essential oils (E.O.s), phenolic compounds, alkaloids, lectins/polypeptides, and polyacetylenes are the main phytochemicals found in plants [13-16].There is mounting evidence that phytochemicals can reduce the accumulation of substances that cause cancer, reduce D.N.A. damage, reduce oxidative cell death, and activate insulin receptors. [17, 18, 19, 20].Phytochemicals have also been reported to generally improve human and animal health [17].

Many plant metabolites display antimicrobial potential against Gram-negative and Gram-positive microscopic organisms [21, 22]. It has been accounted for that the method of activity of these metabolites depends on their capacity to disturb cell walls and cytoplasmic layers, prompting lysis and spillage of intracellular mixtures [23]. In any case, there is restricted factual data about how these mixtures accomplish this antimicrobial action. In addition, there is no data concerning the antimicrobial activity of the tested plant; therefore, we would like to investigate this possibility in our study by utilizing selected medicinal plants. The objectives of the present research were to investigate the antimicrobial and antioxidant properties to exploit the potential use of the Kehail plant.

Methods

Collection of the plant material and microorganisms samples

Fresh specimens of the plant namely *A. decumbens* (Ventenat) Cosson & Kralik (Kehail, Figure 1) were obtained from Jabal Aja (27°259040N, 41°259390E) in the Hail region, during the period (March to May 2021).

Figure 1: Kehail *(A. decumbens*) plant species collected from Aja Mountain (Hail, Saudi Arabia).

Tested bacteria and yeasts

The bacteria tested included: Methicillin-Resistant *Staphylococcus aureus* (217), *Staphylococcus epidermidis* (M13), *Enterobacter cloacae* (155), *Pseudomonas aeruginosa* (M16), *Escherichia coli* ATCC 10536, and *Klebsiella pneumoniae* (140). We obtained

these bacteria previously from King Khalid Hospital, Hail. The microorganisms were maintained on Brain Heart Infusion (B.H.I.) agar medium (HiMedia laboratories, Germany) at 4ºC and re-cultured before using for further analyses. The yeasts tested were *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 9362. All microorganisms were kindly provided by Prof. MejdiSnoussi (Department of Biology, College of Science, University of Hail).

Preparation of plant extract

For the experiment, 20 grams of the plant extract were dissolved in 200 mL of methanol-water (80:20, v/v) in a dark glass container and maintained at agitation for 72h. The extract was then filtered and centrifuged at 5000g for 10 min, and the supernatant was collected and dried to obtain the final crude extract. Mother solution (100 mg/mL) was prepared by mixing 500 mg of the obtained crude methanol-80% extract with 5 ml of dimethyl sulfoxide (DMSO)-5%.

Testing of plant extract for antimicrobial activities

The cup-plate agar diffusion methods were adopted with a few minor adjustments to evaluate the produced extract's antibacterial activity, according to Snoussi et al. [24]. A sterile cotton swab was utilized to inoculate Mueller Hinton (Bacteria) and Sabouraud chloramphenicol (*Candida* species). Petri dishes from a stock suspension containing (10^8-10^9CFU/ml). We incubated the plates at 37°C for 24 to 48 hours. Kehail extract was applied to sterile discs in three distinct dosages (1 mg, 2 mg, and 3 mg/disc). Each concentration was tested against the tested species three times. The diameter of the resulting growth inhibition zones was measured after incubation, averaged, and the mean values were computed. As reference molecules, ampicillin and amphotericin B were utilized.

Microdilution assay was used to determine the minimal inhibitory concentrations values that inhibit the growth of the tested bacterial and fungal strains in the 96-well plates [25]. To stop the growth of the examined microorganisms, the least bactericidal and fungicidal concentrations were calculated. by recording the growth on Mueller-Hinton agar (Bacteria) and Sabouraud Chloramphenicol agar (*Candida* spp.). The tested extract was diluted in DMSO-5%, and serial dilution was prepared in sterile five mL-glass tubes starting from 200 mg/ml to 0.96 mg/mL. Minimal inhibitory concentrations (MICs), Minimal bactericidal concentrations (MBCs), and Minimal fungicidal concentrations (MFCs) values are expressed in mg/mL.

Phytochemical screening

Spectrophotometric assays were employed to access the phytochemical composition of Kehail methanolic extract including phenols, tannins, alkaloids, flavonoids (Alkaline reagent test), terpenoids (Salkowski's test), sterols, cardiac glycosides (Keller-Kiliani's test), saponins, fat, and fixed oils were all examined in the extracts [26-28].

Total Phenolic Content, Total Flavonoid Content, and Total Tannin Content

Determination of total phenolic content (TPC)

The TPC of the Kehail extract was estimated by Kumar et al. [29] method. Folin-Ciocalteu reagent is used. The samples were evaluated at a concentration of 1 mg/mL. 100 L of the extract were put into a test tube, to which 0.75 mL of the Folin-Ciocalteu reagent was added and mixed after being diluted 10 times with deionized water. The mixture was let to stand for 5 minutes at a temperature of 25 °C. The mixture was then gently reacted with 0.75 mL of saturated sodium carbonate solution. A V.-Vis spectrophotometer was used to measure the absorbance at 725 nm following 90 minutes of standing at 25 °C. Using gallic acid, a calibration curve was established. Gallic acid equivalents (G.A.E.) in mg/mg of vegetable extract (mg of gallic acid/mg dry weight) were used to express the overall phenolic content.

Total flavonoid content (TFC)

TFC were determined according to Kumar et al.[29]. A volume of 1.5 mL (1mg/mL) of an identical volume of an extract was added to a 2% AlCl3-6H2O. After vigorously shaking the mixture for 10 minutes, the absorbance at 367 nm was measured. In terms of mg quercetin, the total flavonoid content was stated. /g dry weight (mg Q.E./mg) and determined through a quercetin calibration line.

Total tannin content (T.T.C.)

A modified vanillin assay was used in a colorimetric technique to measure condensed tannins [30]. 50 L of the extract (1 mg/mL) received three ml of a 4% methanolic vanillin solution and 1.5 ml of concentrated H2SO4. After 15 minutes, the mixture was let to stand, and the absorbance at 500 nm was measured with methanol or water used as a reference. The unit of measurement for T.T.C. is mg catechin/g dry weight (mg C.E/mg). All samples were analyzed in three replications.

Determination of antioxidant activity

DPPH assay

DPPH of the extracts and the standard vitamin E were evaluated as reported by the method of Chakraborty and Paulraj [31]. The various extracts (stock solution 20 mg/m and of standard (stock solution 1mg/ml) at different concentrations were pipetted in small tubes. Each sample and standard were combined with an equal volume of DPPH• methanolic solution in a volume of 0.5

mL. The blend was left must stand at a temperature of 25 °C for 30 minutes while it is dark. A spectrophotometer was used to detect the resulting solution's absorbance at 520 nm. As a control, 0.5 mL of DPPH solution and 0.5 mL of methanol were combined. The blank was taken to be pure methanol. The following equation (Eq. 1) calculated the inhibition of DPPH (PI%):

$$PI (\%) = 100 \times (A_{Control} - A_{Sample}) / A_{Control}; \quad \text{(Eq. 1)}$$

Where $A_{Control}$ and A_{Sample} are the absorbances of the control solution and a test sample or standard, respectively.

ABTS radical scavenging activity assay

The 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), also known as the ABTS cation scavenging activity test, was used to conduct an antiradical assay [31]. By treating ABTS solution (7 mM) with 2.45 mM $K_2S_2O_8$, the radical monocation of ABTS was produced. The mixture was let to stand at room temperature for 15 hours in the dark. Samples were dissolved in methanol and distilled water. The varying extract concentrations and the tocopherol (vitamin E). The standard was put to the test, then it was compared to the standard. By mixing 800 mL of diluted ABTS+ with 200 mL of each standard and sample, the antioxidant activity was measured. We measured 30 minutes later, the absorbance measured spectrophotometrically at 734 nm. A triple of each measurement was made. Percent inhibition (%) was used to express the antioxidant capability of the test samples and the reference material. The following equation (Eq. 2) calculated the percentage scavenging of $ABTS^{\cdot+}$ radicals:

$$PI (\%) = 100 \times (A_{Control} - A_{Sample}) / A_{Control}; \quad \text{(Eq. 2)}$$

Where $A_{Control}$ and A_{Sample} are the absorbance of the control and the test sample or a standard, respectively.

β-carotene/linoleic acid method

The method used to assess the -carotene bleaching inhibition was previously disclosed by Ikram et al. [32]. After heating the -carotene/linoleic acid combination, a free radical is produced from linoleic acid. 20 mL of linoleic acid were combined with 200 mL of tween-20, and a total of 2 mL of -carotene solution (1.5 mg -carotene/2.5 mL chloroform) was mixed. The chloroform was removed at 40◦C under vacuum using a rotary evaporator. The dry mixture was mixed with 50 mL of distilled water to create an emulsion of -carotene and linoleic acid. 0.800 mL of the emulsion was mixed to 0.200 mL of extracts at various concentrations (stock solution 20 mg/mL) and the standard (stock solution 1 mg/mL) in order to determine each extract's ability to bleach -carotene. The mixtures were incubated in a water bath at 50°C for 120 minutes, and the absorbance at 470 nm was measured both before and after incubation. The following equation (Eq. 3) was used to calculate the antioxidant activity of extracts:

$$PI\% = [1-(A_0-A_t/A_{c0}-A_{ct})] \times 100; \quad \text{(Eq. 3)}$$

where A_0 and A_{c0} refer to the absorbance values measured at zero time, respectively, for the test sample, the standard, and the control. At an Act, the corresponding absorbance values of the test sample or standard and the control are referred to. These values were measured after incubation for 120 min.

Statistical analysis

All tests were done in triplicate. Mean value and standard deviation were calculated by using the SPSS program for windows, version 20 analyzed data (SPSS Inc., Chicago, IL, U.S.A.).

Results

Antimicrobial activity of Kehail methanolic extract (KME)

The results of the antimicrobial activity of Kehail methanolic extract (K.M.E.) are presented in Table (1). The K.M.E. showed antibacterial activity in vitro against many harmful microorganisms, including Methicillin-Resistant *S. aureus* (217), *S. epidermidis* (M13), *E. cloacae* (155), *P. aeruginosa* (M16), *E. coli* ATCC 10536 and *K. pneumoniae* (140). In contrast, the mean inhibition zone at the lower concentration of the extract (1 mg/disc) was 6.66±0.57mm, 8.88±1.15mm, 11.33±0.57mm, 7.00±0.00mm, 12.00±0.00 mm and 6.00±0.00mm, respectively. On the other hand, the mean inhibition zone at the higher concentration of the extract (3 mg/disc) was 15.00±1.00mm, 14.67±0.57mm, 14.33±0.57mm, 15.00±0.00mm, 15.00±0.00mm and 15.67±0.57mm, respectively. As indicated in Table (1), the highly inhibited bacteria by the plant extract were *K. pneumoniae* (140).Table 1 also indicates that the M.K.E. showed potent inhibition activity against the tested fungi where the mean inhibition zone of *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 9362 at the lower concentration (1 mg/disc) of the plant extract was 13.33±0.57mm and 9.33±0.75mm, respectively, while at the higher concentration (3mg/disc) was16.00±0.00mm and 15.33±0.57mm, respectively. It is evident from the table that the inhibitory effect of the plant extract is greater on fungi than on bacteria, and the higher the concentration of the plant extract, the greater the inhibitory effect.

The results of the determination of MICs, MBCs, and MFCs values of the tested extract against the selective microorganisms were reported in Table (2). The M.I.C. s values ranged from 3.12 to 6.25 mg/mL for the bacteria tested and about 6.25 mg/mL for the two Candida species tested. MBCs values varied from 25 to 50 mg/mL. Using the scheme proposed by Gatsing and colleagues in

Microorganisms tested	*A. decumbnes* methanol-80% extract			Standard molecules*
	1 mg/disc	2 mg/disc	3 mg/disc	
Methicillin-Resistant *S. aureus* (217)	6.66±0.57	12.66±0.57	15.00±1.00	7.33±0.57
S. epidermidis (M13)	8.88±1.15	12.66±0.57	14.67±0.57	24.00 ±0.00
E. cloacae (155)	11.33±0.57	12.66±0.57	14.33±0.57	6.66 ±0.57
P. aeruginosa(M16)	7.00±0.00	13.00±0.00	15.00±0.00	6.00±0.00
E. coli ATCC 10536	12.00±0.00	13.00±0.00	15.00±0.00	10.33±0.57
K. pneumoniae (140)	6.00±0.00	12.00 ±0.00	15.67±0.57	9.33±0.57
C. albicans ATCC 10231	13.33±0.57	14.00±0.00	16.00±0.00	22.61±1.15
C. tropicalis ATCC 9362	9.33±0.75	12.33±1.15	15.33±0.57	14.33±0.57

The inhibition zone around the discs impregnated with A. decumbnes methanol/water extract expressed as means of three replicates (mm±SD). SD: Standard deviation. *: Ampicillin (10 mg/mL; 10 µL/disc) for bacterial strains and amphotericin B (10 mg/mL; 10 µL/disc) for Candida species.

Table 1: The inhibition zone of Kehail methanol/water extract.

Microorganisms tested	*A. decumbnes* methanol-80% extract		Ratio*
	MIC	MBC/MFC	
Methicillin-Resistant*S. aureus* (217)	6.25	50	8
S. epidermidis (M13)	6.25	25	4
E. cloacae (155)	6.25	25	4
P. aeruginosa(M16)	3.125	50	16
E. coli ATCC 10536	6.25	25	4
K. pneumoniae (140)	3.125	25	8
C. albicans ATCC 10231	6.25	25	4
C. tropicalis ATCC 9362	6.25	25	4

* : MBC/MIC ratio, and MFC/MIC ratio are interpreted by using the scheme proposed by Gatsing et al., 2009 [32]

Table 2: Determination of MICs, MBC, MFCs, and ratios of the tested extract against the selected microorganisms expressed in mg/mL.

Saponins	*Terpenes*	*Flavonoids*	*phenols*	*Tannins*	*Alkaloids (Dragendorf)*	*Alkaloids (Mayer)*	*Cardiac glycosides*
+	+	+	+	+	-	-	+

Table 3: Phytochemical profile of *A. decumbens* methanolic extract.

Test System	*Extract*	*(BHT)*	*(AA)*
Phytochemical screening			
Total Phénols (mg GAE/g Extract)	*7.33 ± 0.14*	-	-
Total Tannins (mg TAE/g Extract)	*42.9± 0.25*	-	-
Total Flavonoïdes (mg QE/g Extract)	*58.43 ± 0.74*	-	-
Antioxidant Assays			
1. DPPH IC_{50} (mg/mL)	*0.06 ±3.53*	*0.023±3x10⁻⁴*	*0.022±5x10⁻⁴*
2. ABTS IC_{50} (mg/mL)	*0.17±0.01*	*0.018±4x10⁻⁴*	*0.021±0.001*
3. β-carotene IC_{50} (mg/mL)	*1.9 ± 0.21*	*0.042±3.5x10⁻³*	*0.017±0.001*

BHT: butylated hydroxytoluene; AA: Ascorbic Acid

Table 4: Antioxidant activities of Kehail (*A. decumbnes*) extract as compared to known drugs.

2009, Kehail extract exhibited a bacteriostatic profile against the MRSA strain, *K. pneumoniae* (140), and *P. aeruginosa* (MBC/MIC>4). While the same extract exhibited bactericidal action against *S. epidermidis* (M13), *E. cloacae* (155), *E. coli* ATCC 10536 (MBC/MIC ratio⩽4). For the two *Candida* species, MFC/MIC ratio was about 4, highlighting the fungicidal activity of Kehail extract against these two *Candida* species.

Phytochemical Screening of Kehail methanolic extract

Table 3 summarizes the phytochemical composition of *A. decumbens*. The obtained results indicate that the tested extract contains antioxidants, saponins, terpenes, flavonoids, phenols, tannins, and cardiac glycoside compounds.

Antioxidant Activities

A free radical is characterized as any atom or molecule having unpaired electrons. In living systems, free radicals are produced as a feature of the body's typical metabolic procedure. Antioxidants fight free radicals and protect us from different disorders [33, 34].

Table 4 summarizes the results of the antioxidant activities of Kehail when contrasted with notable molecules as assessed by utilizing DPPH, β- carotene, and ABTS (IC50=mg/mL) scavenging activity. All Khalil extracts showed good radical scavenging activity with an average IC50 value of 0.06+3.53 µg/mL. The ABTS IC50 and β-carotene IC50 averaged 0.17 + 0.01 mg/mL and 1.9 + 0.21 mg/mL, respectively. Acquired outcomes uncover promising antioxidant activities at low concentrations compared to ascorbic acid (A.A.), which was 0.022±5x10-4, 0.021±0.001, and 0.017±0.001 mg/mL, respectively. Furthermore, the obtained values of DPPH IC50, ABTS IC50 , and β-carotene IC50 were greater than those of butylated hydroxytoluene (BHT), which contained 0.023±3x10-4, 0.018±4x10-4 and 0.042±3.5x10-3 mg/mL, respectively.

Discussion

The antimicrobial activity of the plant extract

To evaluatethe antimicrobial capability of the Kehail (*A. decumbnes*), methanol extracts of the whole plant were prepared. The antimicrobial activity was tested against six pathogenic bacteria and two pathogenic fungal strains. Methicillin-Resistant *S.aureus* (217), *S. epidermidis* (M13), *E. cloacae* (155), *P.aeruginosa* (M16), *E. coli* ATCC 10536 and *K. pneumoniae* (140) bacteria and the fungi *C.albicans* ATCC 10231 and *C.tropicalis*ATCC 9362 were used as test strains in the agar well diffusion method. The zone of inhibition was measured to estimate the maximum antimicrobial activity of the plant extract.It has been reported that alcoholic and aqueous extracts of numerous plants and herbs efficiently suppress the growth of potentially harmful food pathogens and rotting microorganisms [35, 36, 8]. There are limited reports of the antibacterial activity of Kehail extract (M.K.E.) against pathogenic bacteria and fungi. As indicated in Table 1, the plant extract showed antibacterial activity against Methicillin-Resistant *S. aureus* (217), *S. epidermidis* (M13), *E. cloacae* (155), *P.aeruginosa*(M16), *E. coli* ATCC 10536 and *K.pneumoniae* (140), whereas the mean inhibition zone was 11.77 ± 0.57 mm, 10.66 ± 0.57 mm, 10.77 ± 0.57 mm, 10.33 ± 0.57 mm, and 9.55 ± 0.57 mm, respectively. It can be well seen from Table 1 that the highly inhibited bacteria by the plant extract was *K. pneumoniae* (140).

In comparison with other studies, some studies indicated that *Arnebia* spp. used in traditional medicine have moderate antimicrobial activities when tested individually, as reported by Abdulameer and Almussawi [37] and Koca et al.[38]. In this study, the inhibition of pathogenic bacteria by *Arnebia* spp. extract is particularly interesting from a medical point of view since the tested bacteria are responsible for severe opportunistic infections. Most crude concentrates display bactericidal action instead of bacteriostatic [39, 40] which essentially features the adequacy of conventional medication.There are a large number of substances that have antimicrobial effects. Still, only a few can be considered therapeutic because mammalian cells are more sensitive to chemical inhibition than microbial cells. It has been previously reported that the extracts from several plants, including *Pergulariatomentosa*L. (Umlebena), *Peganumharmala* L. (Harmal), Roselle (*Hibiscus sabdariffa* L.) *Senna Italica* Mill (Senna), *Teucriumpolium* (Jaâda), and *Carthamustinctorius* (Safflower) other spices had significant ($p< 0.05$) antibacterial and antifungal activities against a variety of pathogenic bacterial and food spoilage bacteria, as well as yeast and mold [8, 41, 42].

Table 1 also indicates that the M.K.E. showed potent inhibition activity against the tested fungi where, the mean inhibition zone of *C.albicans* ATCC 10231 and *C.tropicalis* ATCC 9362 was 12.77 ± 0.57 mm and 13.11 ± 1.52 mm, respectively. The table shows that the plant extract's inhibitory effect is greater on fungi than on bacteria, and the higher the concentration of the plant extract, the greater the inhibitory effect. This study supports past discoveries in the literature that antimicrobial activities have an immediate connection to expanding the concentration of the extracts (%) [8, 42].

The outcomes of this study were comp those acquired by Doulah et al. [43] who found that their tested four Arnebia species namely *A. fimbriopetala*, *A. linearifolia*, *A. garandiflora*, and *A. tubata* could become significant in the acquisition of recognizable sources of mixtures with health defensive potential, cancer prevention agent and antimicrobial activity.

Phytochemical content

The results of the phytochemical examination showed that the samples contained steroids, tannins, terpenoids, saponins, glycosides, and flavonoids. These phytochemicals support the utilization of the Kehail plant in various parts of the Kingdom of Saudi Arabia to prepare traditional medications for treating multiple ailments [3]. These mixtures are biologically active and may add to the antioxidant activities of the *Arnebiadecumbnes*. This plant's extracts may have antioxidant effects on cells since flavonoids and terpenoids are present. Flavonoids apply antioxidant activity through a rummaging or chelating process [44]. Scavenging reactive oxygen species can neutralize lipid oxidation in vitro, develop the body's antioxidant enzyme action, and reduce peroxide arrangement in vivo [45]. Terpenoids decrease the oxidation of lipids by providing radicals with hydrogen, acting as vital antioxidants [46]. Phenolic acids are a significant group of essential antioxidants. They are made of at least one aromatic ring bearing at least one hydroxyl group and can extinguish free radicals by framing stabilized phenoxyl radicals [39]. Due to their abilities to scavenge free radicals, provide hydrogen and chelate metals, halt radical chain reactions, and extinguish singlet oxygen both in vitro and in vivo, polyphenols exhibit strong antioxidant properties [47].

Flavonoids are antioxidants with high redox potentials that allow them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. Ordinarily, flavonoids safeguard plants from infections, oxidative cell damage, herbivores, UV radiation, and fungal parasites. Consistently, flavonoids have been related to a decrease in the occurrence of illnesses like cancer and heart disease [48]. Since the hydroxyl groups

in these mixes are what work to scavenge free radicals, the total amount of phenolic acid and flavonoid content might be used to quickly screen for antioxidant activity. Many investigations have revealed that phenolic acids have other biological activities, for example, possessing anti-inflammatory, ulcer-preventing, spasm-relieving, antiviral, anti-diarrheal, and anticancer activities [49]. Hence, measurement and ensuing identification of phenolic acids can give imperative data connected with the antioxidant capabilities and potential medical advantages of the Kehail plant.

The antioxidant assay

The DPPH, ABTS, as well as β-carotene in testing were utilized to test the antioxidant properties of plant extracts from the Kehail (Table 3). DPPH is a stable free radical, which can promptly encounter a decrease in the presence of an antioxidant, so the lower IC50 value reflected the more potent antioxidant activity. It shows a highly bright and noticeable (UV-Vis) absorbance at 517 nm. The decrease in the power of retention at 517 nm of methanol solutions of DPPH radical in the presence is typically taken as a proportion of their antioxidant activity. The samples' capacity to neutralize the DPPH radical was assessed. based on their concentrations providing 50% inhibition (IC50). Methanol extract of Kehail showed good radical scavenging activity with an average IC50 value of 0.06 + 3.53µg/ml.

The ABTS measure is the most widely recognized strategy to test a compound or plant concentrate's fundamental, radical scavenging activity. The ABTS, produced from the oxidation of ABTS by potassium persulfate, is a magnificent device for deciding the antioxidant activity of hydrogen-donating mechanisms and chain-breaking antioxidants [50]. In this study, the ABTS scavenging activity was expressed as IC50 value to compare the capability of extracting solvents (Table 3). The methanolicKehail extract offered an average value of 0.17 + 0.01mg/L for ABTS IC50. The measurement of ABTS radical scavenging activity has been frequently used in the food industry to measure the antioxidant capacities of foods [50].

The outcomes acquired propose Kehail plant extracts have phytochemicals that can donate hydrogen to free radicals to scavenge them and prevent predicted harm. Plant extracts' phenolic acids are usually linked to their antioxidant properties. The Kehail plant's high total phenolic acid and total flavonoid concentrations are consistent with its high antioxidant activity as shown by the DPPH, ABTS, and -carotene assays. An important class of vital cell reinforcements is represented by plant phenolic acids. They are able to react by producing active oxygen radicals such hydroxyl, superoxide anion, and lipid peroxyl radicals, which can initially suppress lipid peroxidation. The hydroxyl groups in phenolic acids are what give them their strong rummaging properties. Flavonoid and terpenoid antioxidants, which are phytochemicals of interest, are present; additional isolation, purification, and characterization should be done in the future; any isolated compounds may be used to develop novel medications.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Conceptualization, A.S., E.A. and N.A.; methodology, E.A., M.S. and M.H.; formal analysis, A.S., M.A.A., and N.A.; writing-original draft preparation, A.S and M.S.; writing–review and editing, E.A., N.A.; project administration, A.S. All authors have read and agreed to the published version of the final manuscript).

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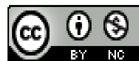

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