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Antimicrobial activities and phytochemical analysis of leaf extracts of *Echinops abuzinadianus* Chaudhary growing in Abha City, Saudi Arabia

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Echinops abuzinadianus belongs to the sunflower or Asteraceae family, which is widely found in an abandoned area in Abha city in Saudi Arabia. The properties of this endemic plant have not been yet researched. This study used agar well diffusion methods to investigatethe antimicrobial inhibition activity of solvent-extracted dry and fresh *E. abuzinadianus* leaves against some human pathogenic microbes. The results show that almost all solvent extracts had significant inhibitory activity against *Shigella flexneri*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Candida albicans.* Fresh leaf-extract had more potent activity against all tested microorganisms than dry leaf-extract. The maximum antibacterial activity against *S. Flexneri* was gained from methanol extract, chloroform extracts against *Pseudomonas aeruginosa*, diethyl ether extracts against *K. pneumoniae* and acetone extract against *C. albicans.* Petroleum ether extract showed moderate antibacterial activities against *P. mirabilis*, while petroleum ether extract exhibited the minimum antibacterial activity against *K. pneumonia*. Gas chromatography mass spectrometry (GC-MS) showed the presence of five phytochemical compounds: palmitic acid (the most dominant compound), followed by 9-octadecenoic acid, octadecatrienoic acid, and trace quantities of octadeca-9,12,15-trienoic acid and phytol. The inhibition of the microbial growth in the presence of solvent extracts of *E. abuzinadianus* leaves and the secondary metabolites produced by the plants, make it a promising medicinal plant.

Keywords: Antimicrobial activities, *Echinops abuzinadianus*, Medicinal plant, Phytochemical analysis, Solvent extracts

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Echinops spp. plants are mainly found in Southeast Europe, North Africa and Southwest Asia[1]. The *Echinops* genus has about 120 species, which are described as a branching perennial that grows up to eighty centimetres. The plant leaves are divided into small and narrow triangular lobes, with a hard needle spine at the tip. The plant annually produces almost spherical flower heads containing many pale lavender, or sometimes white, disc florets but no ray florets[2-6]. *Echinops* spp. plants are common in abandoned land and the adjacent cultivated fields of the Abha area of Saudi Arabia. The plant propagates by seeds, forming small, exclusive scattered communities of the plant. In Saudi Arabia, 10 species of the genus *Echinops* is found namely, *E. abuzinadianus* Chaudhary, *E. glaberrimus* DC., *E. erinaceus* Kit-Tan, *E. macrochaetus* Fresen, *E. hystrichoides* Kit-Tan, *E. Polyceras* Boiss., *E. mandavillei* Kit-Tan, *E. viscosus* DC., *E. sheilae* Kit-Tan, and *E. yemenicus* Kit-Tan. The endemic species in Saudi Arabia are *E. mandavillei*, *E. abuzinadianus*, and *E. sheilae*[7]. In addition to the large number of endemic plant species found in the Saudi Arabia, the flora components are mainly composed of the elements of Asia plants, Africa and Mediterranean region. The greatest species diversity has been recorded in Asir and in Hijaz regions and in the south western area that called Sarawat Mountains in about 2250 species due to plentiful rainfall and the observed altitude range from Tihma region lies at the sea level and up to 9300 ft at El-Sawdah Mountains in Abha City[5]. However, the estimated total flora of Saudi Arabia that showed great medicinal properties expected to be more than 1200 plant species (over 50%) out of its 2250 plant species[8]. Plant-derived natural pharmaceutical products offer a promising source of bioactive agents including, antimicrobial, antivirus and antiparasitic drugs. *Echinops* species plant are widely recorded as being used in folklore medicine to treat many diseases, such as diarrhoea, migraine, heart problems, infectious diseases haemorrhoids and intestinal worm infestations[9]. Additionally, they were used to treat different symptoms such as inflammation, pain and fever. Treat ailments related to respiratory tract

including cough and sore throat was from the other common traditional use of the plant[9]. This research explores the antimicrobial activity of five solvent extracts of *E. abuzinadianus* growing naturally in an abandoned area of Abha city, KSA. The study uses GC-MS analysis techniques to identify active chemical compounds present in the plants.

Materials and Methods

Plant material

Plant leaves were collected from plants growing in Abha city in 2018. Specimens were then stored in a refrigerator at 4℃ in the herbarium of the Biology Department, College of Science, King Khalid University, Saudi Arabia.

Preparation of pathogenic strains

Seven pathogenic human bacterial and fungal strains, *S. flexneri*, *K. pneumoniae*, *P. aeruginosam*, *P. mirabilis*, *S. aureus*, *M. luteus* and *C. albicans*, were first grown in nutrient broth (NB) and incubated at 29°C for 24 h for all bacterial isolates and for 48 h for *C. albicans*[10,11].

Antimicrobial inhibition activity

To examine the antimicrobial activity of different solvent extracts obtained from of *E. abuzinadianus*, the well diffusion method[10] was applied. To start, the density of each fresh bacterial strain was adjusted with sterile distilled water to the McFarland 0.7 standard to arrive at a final concentration of 107 CFU/mL. Then bacterial strains were spread on the surface of sterile Mueller-Hinton agar plates with pH 7.4 at 23°C. Seven grams of ground plant leaves were dissolved in 20 mL the following solvents: methanol, chloroform, petroleum ether, acetone, diethyl ether, hot water (100°C) and in cold water (24°C). Each sample was kept in rotary shaker for 48 h at 200 rpm then transformed to and oven to dry at 59°C for 48 h to allow all solvent to evaporate. To each sample, 7 mL of dimethyl sulfoxide (DMSO) was added then returned to the rotary shaker for 48 h. The extract was filtered using Whitman filter paper and the sample was weighed to a final concentration 1 mg/mL. The well in the agar that had been previously made using a sterile cork borer was filled with 150 uL of each extract. The same volume from was used as the negative control and ampicillin (10 µgdisc^{-1}) was a positive control against Gram-positive and Gram-negative bacteria strains, and *C. albicans*. All plates were kept at 23°C for 3 h and then incubated at 30°C[10]. Each experiment was performed in triplicate. The diameters of inhibition zone were subsequently measured in millimetres.

Phytochemicals analysis

The GC–MS analysis of the *E. abuzinadianus* leaves solvated in methanol extract of was accomplished by using a Clarus 500 PerkinElmer Gas Chromatograph coupled to a mass detector – PerkinElmer Turbo Mass 5.1 spectrometer. The manufacturers' operating instructions were observed. Mass spectra were calculated at 70 eV; the interval scan was 0.5 s and fragments were detected between 40 to 550 Da. Mass spectra results were interpreted by using the database stored in the National Institute Standard and Technology (NIST). The spectrum of the unknown chemicals was characterised by comparing the spectrum of the chemicals present against those stored in the NIST library.

Statistical analysis

SPSS (version 21) and one-way analysis of variance (ANOVA) was applied to determine statistically significant differences between the extracts. A p value of 0.05 was considered statistically significant.

Results

Antimicrobial activity

The antimicrobial inhibition activities of the solvent extract of *E. abuzinadianus* against the seven human pathogenic microbes are presented in Figure 1. It was found that almost all extracts exhibited significant activities against *S. flexneri*, *K. pneumoniae*, *P. aeruginosam*, *P. mirabilis*, *S. aureus*, *M. luteus* and *C. albicans*. In all cases, it was evident that extract obtained from fresh leaves showed 17% greater potency against all tested microorganism than extracts from dry leaves (Fig. 1). No inhibition activity was detected achieved from either cold or hot water extracts. The zone of inhibition for dry leaf-extracts against *S. Aureus* ranged from 1.13 cm (±0.03 cm) for chloroform extract to 1.65 cm (±0.02 cm) for diethyl ether extract; fresh leaf-extract increased to1.66 cm (±0.11 cm) for chloroform extract to 1.90 cm (± 0.05 cm) for diethyl ether. The smallest zones of inhibition were against *S. flexneri;* for dry leaf-chloroform extract, it was 1.06 cm (±0.08 cm) and fresh leaf-chloroform extract was 1.34 cm (± 0.03 cm). Methanol extract showed the greatest antibacterial activity against *S. flexneri*, with an inhibition activity average of 1.40 cm (±0.05 cm) for dry leaves and 1.56 cm (±0.08 cm) for fresh leaves. Chloroform

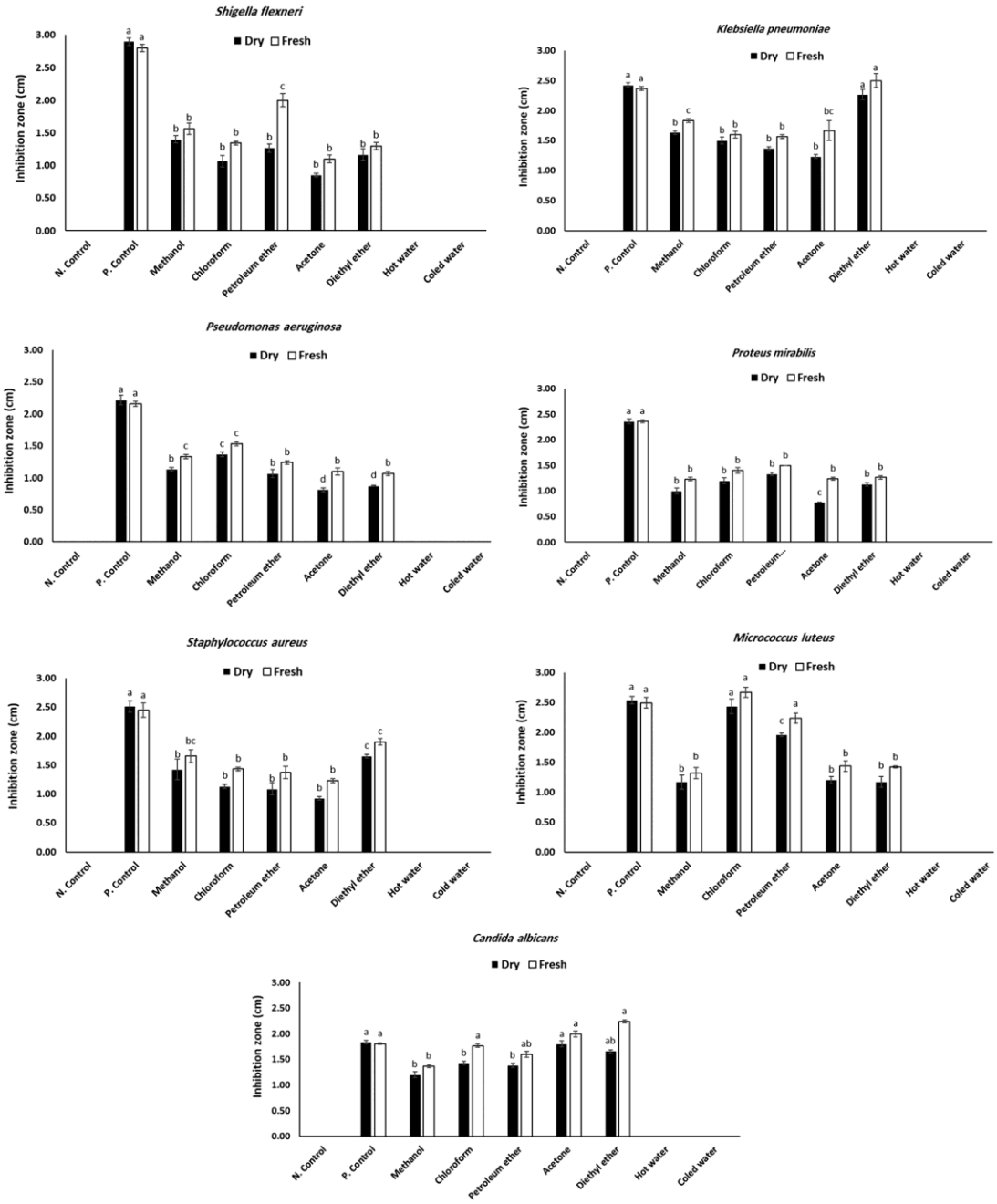

Fig. 1 — Mean values (clearance zones of inhibition (in centimetres)) for antimicrobial activities of dry and fresh leaf extracts of *E. abuzinadianus*. The X-axis represents the different treatments used for plant extraction. Different superscript letters show significant differences between treatments for both dry and fresh plant extracts (One-way ANOVA, Tukey test, $p \leq 0.05$). Error bars represent standard error of the mean for n = 3.

extracts showed the highest antibacterial activities against *P. aeruginosa* with zones of inhibition being 1.36 cm (± 0.03 cm) for dry leaves and 1.53 cm (±0.03 cm) for fresh leaves. Acetone and diethyl ether extracts showed similar activity, respectively averaging 0.81 cm (±0.03 cm) and 0.87 cm (±0.01 cm) for dry leaves and 1.10 cm (±0.05 cm) and 1.06 cm (±0.03 cm) for fresh leaves. Diethyl ether extracts either from fresh or dry leaves showed the strongest antimicrobial activity against *K. pneumoniae* with zones of inhibition averaging 2.26 cm (±0.08 cm) for dry leaves and 2.50 cm (±0.11) cm for fresh leaves. Petroleum ether extract exhibited the lowest antibacterial activity against *K. pneumoniae* with

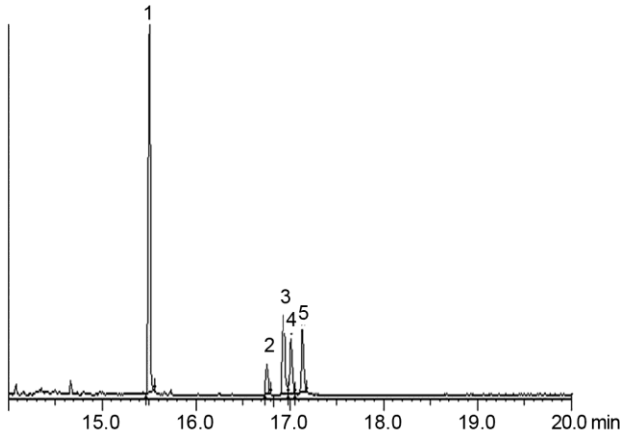

Fig. 2 — Chromatograph of methanol solvent leaf extract of *E. abuzinadianus* plant

Table 1 — GC-MS analysis of methanol leaf extract of *E. abuzinadianus* plant

Compounds	Rt Min.	% Area	M.W.	Ch. Formula
Palmitic acid	15.51	51.01	256.43	$C_{16}H_32O_2$
Phytol	16.76	9.09	296.54	$C_{20}H_{40}$ O
9-Octadecenoic acid	16.94	15.84	282.47	$C_{18}H_{34}O_2$
Octadeca-9,12, 15-trienoic acid	17.02	9.96	278.44	$C_{18}H_{30}O_2$
Octadecatrienoic acid	17.14	14.10	278.44	$C_{18}H_{30}O_2$

zones of inhibition of 1.36 cm (±0.03 cm) for dry leaves and 1.66 cm (±0.16 cm) for fresh leaves. Petroleum ether extract extracts from fresh and dry leaves exhibited moderate antibacterial activities against *P. mirabilis* with an inhibition zone of 1.33 cm (±0.03 cm) for dry leaves to 1.50 cm (±0.00 cm) for fresh leaves, while methanol extracts showed the lowest antibacterial activity of 1.00 cm (±0.05 cm) for dry leaves and 1.20 cm (±0.03 cm) for fresh leaves. *C. albicans* was most susceptible to acetone extract of dry leaves and diethyl ether extracts of fresh leaves, with inhibition zones of 1.88 cm (±0.05 cm) and 2.24 cm (±0.03 cm) respectively. The negative control did not exhibit any antimicrobial activities, while the positive control showed a strong halo indicative of the absence of pathogenic microbes in all plates.

Phytochemical analysis

Characterisation of the active chemicals found in the methanol extracts of dry leaves of *E. abuzinadianus* are presented in Figure 2. GC-MS chromatogram analysis showed five peaks, which indicates the presence of five phytochemical compounds (Table 1). Palmitic acid was the most abundant compound found (51.0%) followed by 9-octadecenoic acid (15.8%) and octadecatrienoic acid (14.0%). Octadeca-9,12,15-trienoic acid and phytol were found in trace quantities of 9.9% and 9.0%, respectively.

Discussion

Natural plant extracts can help to manage multidrug resistant microbes; from ancient times to the present, these substances are often used as potential therapeutic agents for the treatment of many human diseases[12-14]. In this study, well diffusion methods revealed that all solvents with extracts of *E. abuzinadianus* possess antimicrobial activity against all the human pathogenic microbes tested. This reinforces many of the claims for the potential uses of many herbal plants in folklore medicine to treat various diseases and microbial infections. The importance of the ethnobotanical approach is to select specific plants to find new drugs[15-17]. In this regard, the extract of *E. abuzinadianus* could be used to facilitate the synthesis of novel antibiotic drugs, which are urgently needed to solve the problem of multidrug resistant microbes. The microbes used in this study are responsible for many human health problems[18-20]. *Candida* spp. are considered potential human pathogens and the main agent for the mortality rate (63.9%), are responsible for many sexually transmitted infections, such as glans and urinary tract infections. Shigellosis, caused by *Shigella* species, is recognised to a major public health burden; it continues to be an important cause of diarrhoeal diseases. It is estimated that globally, there are around 164.7 million episodes of *Shigella* annually; of these, 163.2 million occur in developing countries, resulting in about 1.1 million deaths[21]. In underdeveloped and in developing countries that have poor sanitary conditions, where transmission from person to person is easy, or when water or food is contaminated by the microorganism, epidemics are generally common[22]. *K. pneumoniae* is responsible for a number of infectious diseases, ranging from pneumonia to urinary tract infections representing a major healthcare threat[23]. *Proteus* spp. bacterial strains are commonly known to be opportunistic human pathogens. Their pathogenic mode of action and their virulence factors enable the bacteria to thrive in different niches of the host organism[24-27]. *P. aeruginosa* has been reported to be as an opportunistic Gram-negative bacterial strain, representing a main cause of morbidity and mortality in people with cystic fibrosis (CF), ventilated patients, chronic obstructive pulmonary disease (COPD), and patients subjected to immunosuppression[28,29]. *S. aureus* is considered a

human opportunistic pathogenic bacterial strain, which causes a variety of infections that affect a range of tissues and organs including skin, soft tissue, bloodstream, joints and bone, as well as causing pneumonia[30,31]. *M. luteus* bacterial strain is known to cause pneumonia in immunocompromised patients; it is also involved in skin infections and can be found in a wide range of adverse environmental conditions, including ultraviolet exposure and chemical contamination[32]. This research showed there was significant variation in the antimicrobial activity of the different solvent extract against investigated pathogenic microorganism supporting the polarity and non-polarity of the applied solvents. Other works using both the agar well diffusion method and the agar tube dilution method to evaluate the antimicrobial activity of methanol, ethanol and aqueous extracts of *Ricinus communis* plant, found that the extract efficiency depend mainly on the type of solvent used; however, the methanol leaf extracts had significant inhibition against pathogenic strains compared to other leaf extracts[33]. The *in vitro* antibacterial activity of various solvents and water extracts of some plant were assessed on some multi-drug resistant bacterial strains found that the zone of inhibition varied greatly depending mainly on the type of plant extract, the type of solvent used for extraction, and the type organism tested[34,11]. In this study, extracts obtained from fresh leaves demonstrated stronger inhibition activity than extracts from dry leaves, indicating that drying the plant material may cause loss some of bioactive chemicals. This finding is consistent with other studies[35,36]; for example, Alrumman[37] assessed the antimicrobial activities of dry and fresh leaves of *Aloe vacillans* against clinical isolates of human pathogens and found the zone of inhibition for fresh-leaf extracts was greater than dry-leaf extracts.

All of the compounds identified by GC-MS are considered to offer biological benefits. For example, phytol compounds have been shown exert pronounced antinociceptive actions in nociception models, as well as found to beantioxidant[38]. Palmitic acid (PA) is a saturated fatty acid and accounts for 20%–30% of total fatty acids in the human body; it is reported to play an important role in the two classes of protein fatty acid acylation, side-chain palmitoylation and N-terminal myristoylation[39]. It also has a negative side effect of increasing pro-inflammatory responses in immune cells through Toll-like receptor 4 and many other side effects. Octadecatrienoic acid derivatives found in *Brassica nigra* L. (Brassicaceae) have antimicrobial activity against a range of pathogenic microorganisms[40].

Conclusion

In conclusion, the results demonstrated that the extract of *E. abuzinadianus* naturally possesses antimicrobial inhibition activity that could be considered an alternative source of antibiotics. Additionally, the plants have many useful biological chemicals that could be used in various pharmaceutical purposes. Further research should be done to elucidate the mode of action of the active chemicals and determine the potential of the plant's toxicity to humans.

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Conflict of Interest

The author has no conflict of interest to declare.

Author's Contributions

The author endorses accountability of the manuscript preparation from planning and accomplishment.

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