

Original Research Article

Phytochemical, antimicrobial and cytotoxicity screening of ethanol extract of *Acacia ehrenbergiana* Hayne grown in Jazan Region of Saudi Arabia

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Abstract

Purpose: To explore the phytoconstituents of *Acacia ehrenbergiana* Hayne as well as its biological effects.

Methods: Determination of phytoconstituents of ethanol extract of the plant was performed by gas chromatography-mass spectrometry (GC-MS) technique. Antibacterial screening was conducted against the isolates of Gram-positive and Gram-negative microbes while the anti-carcinogenic properties of the ethanol extract on cancerous cells were investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay against breast MCF7, ovary cancer A2780 and colon cancer HT29 cells, respectively, in addition to normal MRC5 fibroblast cells.

Results: GC-MS analysis identified 15 different phytochemicals in the ethanol extract. The extract exerted significant antimicrobial activity with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in the range 1.56 - 6.25 and 3.12 - 12.5 mg/L, respectively, against all test bacterial strains. Cytotoxic activity, obtained by MTT assay, was 28.81 ± 0.99 , 12.50 ± 2.50 , 23.90 ± 0.74 and 50.58 ± 3.24 $\mu\text{g/mL}$, against the three cancer cell lines and normal fibroblast, respectively. MTT cytotoxicity results was further confirmed by clonogenic survival assay on MCF7 cells.

Conclusion: This study highlights the potential interesting ethnopharmacological applications of *Acacia ehrenbergiana* Hayne to treat drug-resistant pathogens as standardized extract.

Keywords: *Acacia ehrenbergiana*, Phytochemistry, Antimicrobial, Cytotoxicity

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INTRODUCTION

Acacia ehrenbergiana Hayne (Fabaceae) is commonly found in the deserts of East Africa,

northern Sahel, Arabian Peninsula, Southern and Central Sahara and is locally known as Salam. Higher salinity, pH and temperature tolerance makes this plant appropriate for the

environmental conditions of Saudi Arabia [1,2]. Globular golden yellow fluffy flowers blossom in March and are about 1.5 cm wide which are visited by bees to make Acacia honey with high phenolic antioxidants [2,3]. Different acacia species have been used in folklore medicine and have high therapeutic values. Previously, ethanol extract of matured leaves showed significant anti-inflammatory activity [4], whereas, the polar and non-polar extracts of different parts of the tree (seeds, fruits, stem bark, leaves) showed effective antibacterial activity with no activity against most of the fungal species [5,6]. A 3 % Stem bark ointment of the tree possessed complete wound healing and antibacterial activity as compared to tetracycline after 11 days of treatment in rats [7]. Salam from north and east Africa showed presence of Gallic acid, rutin, myricetin, methyl gallate, rutinoid, quercetin, and catechin [8]. Another species isolated from Aswan, Egypt showed presence of new acylated flavonol diglycoside, rutinoid (1), gallic acid (2), methyl gallate (3), quercetin-glucopyranoside (4), myricetin-*D*-glucopyranoside (5), rutin (6), myricetin-*D*-glucoside (7), quercetin-*D*-glucoside (8), myricetin (9), quercetin (10), catechin (11) in the aerial parts. Compounds 1, 2, 3, 5 and 10 illustrated good antibacterial and antifungal activity [8-11]. There is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from other non-synthetic sources, such as plants owing to the multiple drug resistance and adverse effects of currently used antibiotics [9].

Flavanoids, alkaloids, glycosides, tannins and saponins were also reported to be present in the plant collected from Saudi Arabia [6]. Till date, no data is available on the phytochemical constituents of *Acacia ehrenbergiana* Hayne locally grown in southern Saudi Arabia. Since, the constituents of the plant differ significantly from one region to another; this study was intended to explore the phytoconstituents using GC-MS and to test its antimicrobial and anti-carcinogenic properties on breast, ovary and colon cancer cells, by MTT cytotoxicity assay.

EXPERIMENTAL

Plant material

Yellow flowers of *A. ehrenbergiana* Hayne were collected from Jazan in the month of March 2018 and were identified by Yahiya Masruhi, Department of Botany, Faculty of Science, Jazan University, Saudi Arabia. A voucher specimen no. JU/COP/18-1 was deposited in the departmental herbarium.

Preparation of extracts

Flowers were dried at room temperature and were used to prepare the extracts. Dried flowers (100 g) were milled and homogenized at 5000 rpm with 300 mL ethanol thrice for 24 h. The combined extracts were mixed, filtered through Whatman paper (0.45 μ m), and dried under reduced pressure to obtain a dark colored mass. The residue thus obtained was kept at 4 °C in the dark until GC-MS analysis was performed.

GC-MS analysis of the extract

The flower extract suitably diluted in methanol (1:10 v/v) was analyzed using Thermo Scientific GC-MS instrument equipped with AS 3000 auto sampler; trace ultra GC and ISQ detector. Thermo Scientific TR 5MS with dimensions of 30 m \times 0.25 mm (internal diameter) \times 0.25 μ m (film thickness) was used for the separation of components. Helium, at a flow rate of 1.2 mL/min (constant flow mode), was used as carrier gas. A volume of 2 μ L of sample extract was injected to the system in split less mode using auto sampler. The injection port was set at 320°C and the oven temperature was initially set at 70 °C for 5 min, which was subsequently ramped to 205, 280, 290 and finally to 300 °C at a rate of 5 °C/min and held for 5 min at each temperature. The maximum oven temperature was set at 320°C. The mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 60 - 900 amu with 0.6 scan times (min). The MS transfer line temperature and ion source temperature were kept at 320 and 350 °C respectively with electron multiplier voltage of 1 Kv.

Identification of constituents

The mass spectra were interpreted using X-caliber software and the fragmentation patterns obtained for different components were matched with the structures stored in the database using MAINLIB, NIST and REPLIB built-in libraries. The constituent percentages were measured on the basis of peak area and the identification of components was based on comparison with compounds available in computer library (NIST and Willey) associated with the GC-MS instrument.

Antimicrobial assays

Test microorganisms

The extract was tested against three species of Gram positive bacteria, *Staphylococcus aureus* (ATCC 254996), *Enterococcus faecalis* (ATCC

254602) and *Streptococcus pneumoniae* (ATCC 254657) as well as four species of Gram negative organisms, *Escherichia coli* (ATCC 254607), *Proteus mirabilis* (ATCC 257440), *Pseudomonas aeruginosa* (ATCC 254992), *Klebsiella pneumoniae* (ATCC 254656) and one standard fungus, *Candida albicans* (ATCC 254625). The tested organisms were obtained from the Department of Microbiology, King Fahad Hospital, Jazan, Saudi Arabia.

Preparation of the test organisms

A loopful of isolated bacterial colonies was inoculated into 4 mL peptone water and allowed to incubate for 4 h at 37 °C. The turbidity of actively growing bacterial suspension was adjusted and matched with the standard turbidity of 0.5 Mc Farland units [12]. The fungal cultures were retained on Sabouraud dextrose agar medium and incubated for 4 days at 25 °C. The resultant fungal suspension was then harvested and washed with 100 mL sterile normal saline solution and stored in refrigerator at 4 °C until used further.

In vitro antimicrobial screening of extract

To test the antibacterial activity of the plant extracts, cup plate agar diffusion method were adopted with some minor modifications [13]. In this method, 1 mL of the standardized bacterial stock suspension containing 10^5 - 10^6 C.F.U/ mL was thoroughly mixed with 100 mL of Muller Hinton agar medium, kept at 45 °C. Aliquots of 20 mL of the resulting Muller Hinton agar media were distributed into sterile Petri-dishes and kept aside to set. Four cups of approximately 20 mm diameter were made in each of these plates using sterile cork borer number 4. Agar discs were removed and the holes were filled with 0.1 mL of plant extracts and allowed to get diffused at room temperature for minimum 2 h. All these plates were kept in incubator for 18 h at 37 °C temperature. All the experiments were carried out in duplicate and the diameters of resulting zone of inhibition were measured and the mean values were reported. Similar procedure was adopted for testing the extract for antifungal activity. Instead of Muller Hinton agar, Sabouraud-dextrose agar medium was utilized and the incubation was performed for 2 days at 25 °C in case of *Candida albicans* fungi.

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) was determined for the crude extract of *Acacia ehrenbergiana* Hayne against the

microorganisms using broth microdilution method. Test bacterial cultures (100 µL of bacterial culture containing 10^5 CFU/mL) were inoculated into tubes containing different concentrations of extract of 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 mg/L and incubated for 24 h at 37 °C. The values were determined by detecting the inhibition of visible growth in the culture tubes. Similarly, Minimum Bactericidal Concentration (MBC) was measured by subculturing the broth onto freshly prepared solid media and further incubated at 37 °C for 24 h. The least concentration of MIC tubes which did not reveal any sign of growth of bacterial colonies was regarded as MBC [14].

Morphological analysis

A thin film of treated bacterial cell solutions of *S. aureus*, *E. coli* and *P. aeruginosa* were inoculated with 50 µL of the *A. ehrenbergiana* solution (1 mg/L) and were smeared on a silver stub for SEM analysis [15,16]. All the samples were coated with gold using cathodic spraying (Polaron gold) technique and were dried under mercury lamp for 5 min. The morphological changes in the microorganisms were assessed by scanning electron microscope after 18 and 24 h of the inoculation.

Cytotoxicity assay

Three cancer cell lines, A2780 (human ovary adenocarcinoma), MCF7 (human breast adenocarcinoma) and HT29 (human colon adenocarcinoma) were used in this study, along with MRC5 (normal human fetal lung fibroblast) cells. All cells were obtained from the ATCC. The three cancer cell lines were sub-cultured in RPMI-1640 media (in 10 % Fetal Bovine Serum (FBS); whereas, MRC5 was cultured in Eagles minimum essential medium (EMEM, in 10 % FBS); kept at 37 °C, 5 % CO₂ and 100 % relative humidity).

The cytotoxicity of the extracts were evaluated using MTT assay following the procedure described previously [17]. The three cancer cell lines and one normal fibroblast were separately cultured in 96-well (3×10^3 /well) plate and incubated at 37 °C overnight. Different plant extract concentrations of 0, 6.25, 12.5, 25, 50, 100 µg/mL were prepared in DMSO (0.1%; n = 3), added to the plates and incubated for 72 h, followed by addition of MTT to each well. Plates were incubated further for 3 h, supernatant was aspirated followed by addition of DMSO to each well. Absorbance was measured on a multi-plate reader. Optical density of the purple formazan A₅₅₀ was considered to be proportional to the

number of viable cells. Compound concentration causing 50 % inhibition (IC₅₀) compared to control cell growth (100 %) was determined.

Clonogenic assay

Clonogenic assay is used to measure survival of tumor cells and their subsequent proliferative ability upon exposure to drugs [18,19]. Exponentially growing MCF7 cells in DMEM (previously supplemented with 10% FBS + 1 % Penicillin/Streptomycin) media were seeded in duplicates at a density of 200 cells per well in a six-well plate and allowed to get attached for overnight. The cells were then exposed to increasing concentrations of ethanol extract (0, 12.5, 25 and 50 µg/mL) of plant and incubated for 72 h. Wells containing media with extract were replaced with fresh media without extract. Cells were left to grow at 37 °C, 5 % CO₂ and 100 % humidity. Wells were checked every day and cells forming colonies were roughly counted. After incubation for 14 days, the plates were rinsed thoroughly using PBS and fixed with chilled methanol for 20 min at room temperature. The plates were then stained with 0.5 methylene blue solution prepared in 1:1 methanol/H₂O (v/v) for 10 min, rinsed well using distilled water and finally dried in air. Cell colonies were counted macroscopically and reported.

Statistical analysis

Data were statistically analyzed using SPSS (IBM Inc, USA) version 20 software and are

reported as mean ± standard error of mean (SEM). Differences between groups were accordingly assessed by inferential statistics. *P* < 0.05 was considered statistically significant.

RESULTS

Phytochemical composition of plant extract

The phytochemical compounds identified in *A. ehrenbergiana* Hayne ethanol extract of flowers are tabulated in Table 1. These components are arranged in order of GC elution on TR-5MS column. Total 57.67 % of phytoconstituents were calculated from ISQD chromatograms and relative concentration of each compound in the extract was quantified according to the peak area integrated by the analysis program. The extract was characterized by a large number of higher alkanes (10.11 %), glycerides (18.85 %), caroteneoid pigments (2.66 %), 9,15-diazatetracyclo-hexadeca-tetraen-11-yl) methyl acetate (5.47%), propanoic acid ester (1.01 %), oxaspiro(4,5)deca-6,9-diene (4.25 %), phytosterol (1.76 %), azulene derivative (1.93 %) and acetic acid derivatives (1.66 %). Heptacosane (2.29 %), 2-methyl- nonadecane (2.77 %), 2-methyl-eicosane (1.16 %), 3-ethyl-5-(2-ethylbutyl)-octadecane (2.66 %) and tetratetracontane (1.51 %), were different derivatives of higher alkanes found in the flowers.

Table 1: Phytochemical constituents of ethanol extract of the flowers of *A. ehrenbergiana*

S. no	Retention time	Chemical constituent	MW	Molecular formula	% Area
1	5.62	Dasycarpidan-1-methanol, acetate (ester)	326	C ₂₀ H ₂₄ O ₅	5.47
2	18.88	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	216	C ₁₂ H ₂₄ O ₃	1.01
3	31.25	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	C ₁₇ H ₂₄ O ₃	4.25
4	31.71	Heptacosane	380	C ₂₇ H ₅₆	2.29
5	32.07	2-methyl- Nonadecane	282	C ₂₀ H ₄₂	2.77
6	37.0	2-methyl- Eicosane	296	C ₂₁ H ₄₄	1.16
7	40.48	1,8-Diazacyclotetradecane	226	C ₁₂ H ₂₂ N ₂ O ₂	2.38
8	42.58	3-ethyl-5-(2-ethylbutyl)- Octadecane	366	C ₂₆ H ₅₄	2.66
9	43.35	Tetratetracontane	618	C ₄₄ H ₉₀	1.51
10	53.09	1-Monolinoleoylglycerol	498	C ₂₇ H ₅₄ O ₄ Si ₂	18.85
11	56.41	Lycopene	536	C ₄₀ H ₅₆	0.78
12	59.06	1,1',2,2'-tetrahydro-1,1'-dimethoxy-Carotene	600	C ₄₂ H ₆₄ O ₂	1.88
13	60.76	β-Sitosterol	414	C ₂₉ H ₅₀ O	1.76
14	66.59	4a,5,7b,9,9a(1aH)-pentol,3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-5,9,9a-triacetate,1H-cyclopropa[3,4]benz[1,2-e]azulene	534	C ₂₈ H ₃₈ O ₁₀	1.93
15	67.83	17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-acetic acid	534	C ₃₃ H ₅₅ ClO ₃	1.66

Two carotenoids (tetraterpenoid), lycopene (0.78 %) and 1,1',2,2'-tetrahydro-1,1'-dimethoxycarotene (1.88 %) were also present which were responsible for the yellow color of the flower. Individually identified constituents from flowers collected from Jazan were 1-monolinoleoylglycerol (18.85 %), β -sitosterol (1.76%), 4a,5,7b,9,9a(1aH)-pentol and 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate,1H-cyclopropa[3,4]benz[1,2-e]azulene (1.93 %) derivative. The structures of some important components present in ethanol extract are given in Figure 1.

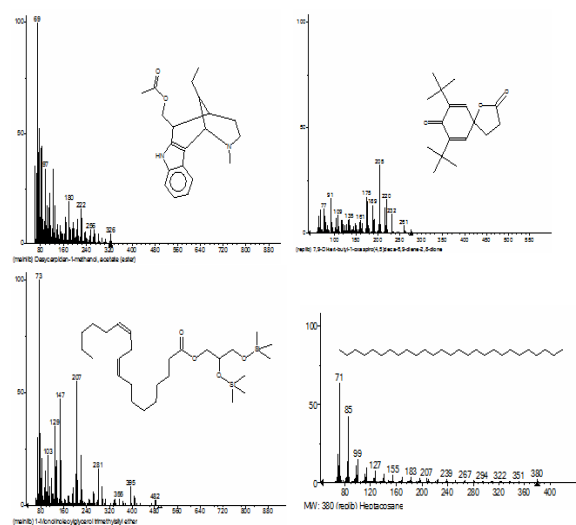


Figure 1: Structure and mass spectra of some major chemical components detected in *A. ehrenbergiana*

Antibacterial activity

The antibacterial efficacy of ethanol extract of *A. ehrenbergiana* against bacteria was examined by the disc-diffusion method and the results are shown in **Table 2**. Plant extract was found to be effective against the isolates of Gram-positive microorganisms, *S. aureus* and *E. fecalis* as well as Gram-negative organisms, *E. coli*, *P. aeruginosa*, *P. mirabilis* and *K. pneumonia* which were resistant to one or more commercially available antibiotics. The diameter of zone of inhibition was found to be in the range of 15 – 22 mm against various bacterial strains tested, with maximum diameter against *S. aureus* (22 ± 0.3 mm) bacteria. Antibacterial activity measured by the disc-diffusion method has some limitations as it only indicates the bacterial growth inhibition without any evidence of the bacteriostatic or bactericidal action of the extract. Therefore, determination of MIC and MBC values of extract is used to establish the dose specificity and nature of the activity of the extract. Minimum inhibitory concentration (MIC) values of *A. ehrenbergiana* were estimated using broth

macro-dilution method, whereas, MBC values were determined by sub-culturing all prepared concentrations (\geq MIC) with no detectable growth. The MIC and MBC values of *A. ehrenbergiana* were calculated to be in the range of 1.56 - 6.25 mg/L and 3.12 – 12.5 mg/L, respectively against all bacterial strains tested as shown in Table 2.

Table 2: Diameter of zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *A. ehrenbergiana* ethanol extract

Microbial strain	MBC (mg/L)	MIC (mg/L)	Zone of inhibition diameter (mm, Mean ± SEM)
DMSO	-	-	-
<i>Staphylococcus aureus</i> (ATCC 254996)	3.12	1.56	22 ± 0.3
<i>Enterococcus fecalis</i> (ATCC 254602),	12.5	6.25	18 ± 0.2
<i>Streptococcus pneumonia</i> (ATCC 254657)	^a	-	-
<i>Escherichia coli</i> (ATCC 254607)	3.12	1.56	15 ± 0.1
<i>Klebsiella pneumoniae</i> (ATCC 254656)	-	-	-
<i>Pseudomonas aeruginosa</i> (ATCC 254992)	6.25	3.12	18 ± 0.2
<i>Proteus mirabilis</i> (ATCC 257440),	12.5	6.25	18 ± 0.4
<i>Candida albicans</i> (ATCC 254625).	-	-	-

^aNo activity observed at the test concentrations

The effects of *A. ehrenbergiana* on the membrane morphology of *E. coli* reference strain ATCC 254607, *S. aureus* reference strain ATCC 254996 and *P. aeruginosa* reference strain ATCC 254992 were demonstrated using Scanning Electron Microscopy (SEM). Untreated bacterial cells showed normal intact outer cell membrane and retained their morphology. Following incubation with *A. ehrenbergiana* at concentration 1 mg/L, physical damage and irregularities and rupture were observed on the cell membrane of treated bacteria.

The treatment of bacteria with the extract of *A. ehrenbergiana* also resulted in partial deformation of the cell wall and formation of cavities in cells as well as shrinkage, aggregation and ultimately ruptures. SEM images of tested microbial strains after the treatment showed distorted shapes and lost of integrity of bacterial

cells after 18 and 24 h of incubation as depicted in Figure 2.

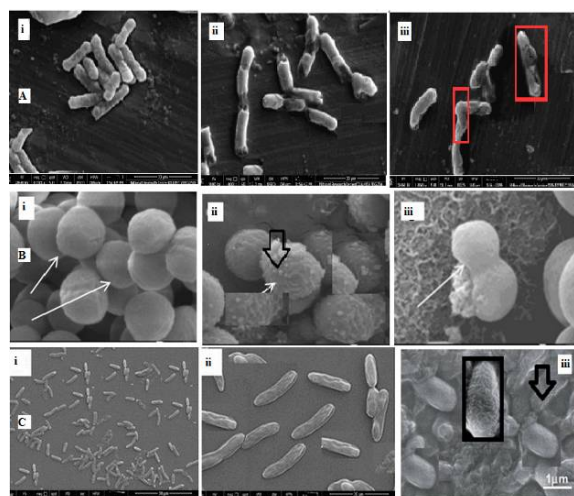


Figure 2: Morphology of A) *E. coli*, B) *S. aureus* and C) *P. aeruginosa* bacterial cells observed by Field Emission Scanning Electron Microscopy. (i) untreated cells (control), (ii) treated cells with 1 mg/L concentration of *A. ehrenbergiana* for 18 h and (iii) treated cells with 1 mg/L concentration of *A. ehrenbergiana* for 24 h. The surface damage and adsorption of *A. ehrenbergiana* on the bacterial cell is marked

Cytotoxicity and clonogenic assays

The cytotoxic activity (MTT 72 h, $IC_{50} \pm SD$ $\mu\text{g/mL}$) of the ethanol extract of *A. ehrenbergiana* Hayne against three cancer cell lines MCF7, A2780 and HT29 as well as against normal fibroblast cells, MRC5 was found to be 28.81 ± 0.99 , 12.50 ± 2.50 , 23.90 ± 0.74 and 50.58 ± 3.24 $\mu\text{g/mL}$, respectively (Table 3).

Table 3: Cytotoxic activity of the ethanol extract of *A. ehrenbergiana* against three cancer cell lines and one normal fibroblast (MTT 72 h, $IC_{50} \pm SD$ $\mu\text{g/mL}$)

Cell line	$IC_{50} \pm SD$ ($\mu\text{g/mL}$)
MCF7	28.81 ± 0.99
A2780	12.50 ± 2.50
HT29	23.90 ± 0.74
MRC5	50.58 ± 3.24

Clonogenic survival assay was performed to test the clonogenic effect of ethanol extract on MCF7 cells. Cells were observed after 72 h of exposure to the ethanol extract, followed by 14 days incubation in fresh nutrient medium. The plant extract inhibited the growth of MCF7 colonies (**Figure 3**) in a concentration dependent manner. The result of the clonogenic survival assay further supported the MTT results reported in this study.

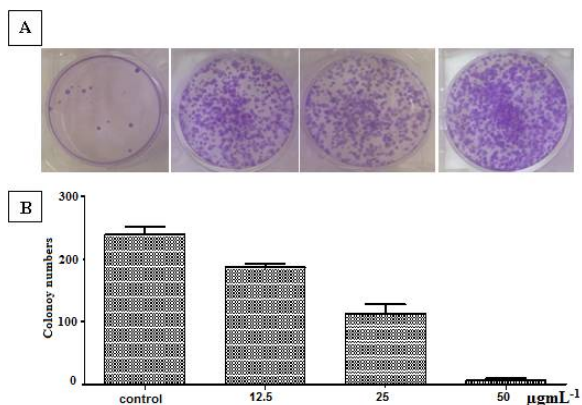


Figure 3: Clonogenic results. A: Colonies of MCF7 treated with ethanol extract (from left 0, 12.5, 25 and 50 $\mu\text{g/mL}$ concentration; $n = 3$) in 6 well plates following 14 days of extract-free incubation. B: bar graph showing ethanol extract concentrations (X-axis) and colony numbers (Y-axis). Results are expressed as cell number \pm SD of three independent experiments

DISCUSSION

Using GC-MS, 56.09 % phytoconstituents from ethanol extract were identified. Components such as dasycarpidan-1-methanol acetate (ester), β -sitosterol, 1-monolinoleoylglycerol, squalene, octacosanol, α -amyirin and lupeol have been previously reported to have antibacterial, antifungal, anti-inflammatory, anti-pyretic, anti-diabetic and anticancer activities [20-22].

Other important detected constituents including 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, heptacosane, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-carotene, lycopene and 2-methyl-eicosane are well known antioxidants. On the other hand, 2-methyl-eicosane, 3-ethyl-5-(2-ethylbutyl)-octadecane, 1-heptacosanol, tetratetracontane, and 2-methyl-nonadecane are known antimicrobial agents [23,24]. Among the antioxidant components, ζ -tocopherol, squalene, β -sitosterol, hentriacontane, heptacosane, 2-methyl-eicosane, octacosane, heneicosane were the most abundant ones which are produced by mature flowers of the plant [25,26].

Plants are one of the major sources of naturally occurring antibacterial compounds [27]. Development of newer antimicrobial agents is urgently needed owing to the harmful side effects and antibiotic resistance associated with the existing antimicrobial compounds. Therefore, medicinal plants have now been evaluated as newer antibiotics owing to their potent activity. The study was aimed to investigate the antimicrobial efficacy of locally grown *A. ehrenbergiana* Hayne (Salam) species extracts as a grazing herb against some medicinally significant pathogens. Three Gram positive

standard bacterial strains (*S. pneumoniae*, *S. aureus* and *E. fecalis*) and four Gram negative standard bacteria; strains (*E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa*) as well as one standard fungus (*Candida albicans*) were tested.

The results obtained are summarized in Table 2 and they show that ethanol extract of the plant exhibited significant antimicrobial activities against most of the pathogens including *S. aureus*, *E. fecalis*, *E. coli*, *P. aerogenes* and *P. mirabilis* as demonstrated by agar well diffusion method. However, the extract did not reveal any significant antifungal efficacy against the tested fungal strain *C. albicans*. These results were in concordance with the results obtained by El-Desoukey *et al* [6]. and Rahim *et al* [7], who reported that the methanol and aqueous extract of *A. ehrenbergiana* displayed a wide range of activity when tested against a number of bacterial strains with promising MIC values. The methanol extract of *A. ehrenbergiana* was explored for its antimicrobial activity, which displayed a wide spectrum of activity against a number of bacterial isolates with a weak inhibitory action on tested fungal strains. In contrast, Younis *et al* [28] reported that the aqueous, ethanol and ethyl acetate extracts of *A. ehrenbergiana* shoots showed moderate antimicrobial activity.

The presence of biologically active constituents such as saponins, alkaloids, flavonoids and tannins in leaves and flowers in varying concentrations might be responsible for the antibacterial efficacy of *A. ehrenbergiana* [28]. The antimicrobial action of tannins obtained from the plant has been demonstrated through coagulating the protoplasm of the microorganism [28]. Biological functions of flavonoids include removal of beta toxins, reduction of inflammation, protection against allergies, platelet aggregation, treatment of ulcer, scavenging free-radicals, killing microbes and treatment of tumors [6,29]. The MIC values as shown in Table 2 were observed to be lesser in the current study as compared to the previously reported [30] values.

The results obtained in the microscopic studies are in agreement with the previous report, demonstrating irreversible damage which greatly occur to bacterial membranes, leading to loss of the most essential cellular components such as nucleic acids and proteins after the treatment with the plant extract [31]. To evaluate the antibacterial action, it is necessary to investigate the changes in morphology, surface characteristics, membrane permeability and integrity of the bacterial cell before and after

treatment with extract [31]. The morphological and physiological changes in *E. coli*, *S. aureus* and *P. aeruginosa* (Figure 2 A - I) were observed by scanning electron microscopy (SEM) technique before and after treatment with *A. ehrenbergiana* extract. Results showed obvious and direct destructive effects of the extract on tested organisms and distinct morphological changes as compared to untreated ones. Most of the treated bacterial cells appear broken, pitted and deformed after 18 and 24 h of the treatment. These investigations supported the results of integrity and cell permeability assay and ensured that the *A. ehrenbergiana* extract had major effects on the cytoplasmic membrane and cell wall of bacteria.

CONCLUSION

A total of 15 phytoconstituents have been identified from the ethanol extract of *A. ehrenbergiana* Hayne grown in the southern part of Saudi Arabia. The extract exhibits promising antimicrobial effects against several bacterial strains including *S. aureus*, *E. fecalis*, *E. coli*, *P. mirabilis*, *P. aeruginosa* and *K. pneumoniae*. This extract also has potential anti-carcinogenic activities against breast, ovary and colon cancer cell lines. Further preclinical and clinical studies are required to elucidate the exact mechanism of antimicrobial and cytotoxic activities of the plant.

DECLARATIONS

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Conflict of interests

The authors declare that no conflict of interest, financial or otherwise, is associated with this work.

Contribution of authors

This work was done by the author(s) named in this article and all liabilities pertaining to claims and contents will be borne by the authors. Hafiz A. Makeen and Hassan A. Alhazmi carried out the conceptualization and design of the study and experimental design. Asaad Khalid, Mohammed Al Bratty, Sohier M. Syame, Ashraf N. Abdalla, and Husham E. Homeida carried out the experimental work and data analysis. Shahnaz Sultana and Waquar Ahsan wrote and

proofread the manuscript. All the authors read and approved the manuscript for publication.

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