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Phytochemical screening, and *in vitro* antimicrobial potential of *Aerva javanica* leaf extracts, collected from Shada Mountain, Al-Baha, Saudi Arabia

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Abstract

The purposes of this study were to determine the phytochemical constituents and *in vitro* antimicrobial efficacy of *Aerva javanica* (L.) leaf extracts growing wildly in Al-Baha region, Saudi Arabia. The plant leaves were collected, air-dried, macerated, and then extracted with ethanol, chloroform and hot water. The phytochemical constituents and antimicrobial potential against Gram-positive bacteria including; *Staphylococcus aureus*, *Bacillus cereus*, Gram-negative bacteria such as; *Escherichia coli*, *Pseudomonas aeruginosa*, and the yeast fungus *Candida albicans* were determined. Results indicated that the extracts contained saponins; coumarins, alkaloids, tannins, flavonoids and steroids. Gas chromatography-mass spectrometry (GC/MS) analysis revealed the presence of 38 different compounds in the ethanol extract, 41 compounds in chloroform extract, and 27 compounds in the aqueous extract. As the concentration of the ethanol extract increased from 25 mg/ ml to 300 mg/ ml, the *in vitro* antimicrobial potency against the tested microorganisms increased. At 50 mg/ ml, the extract was inactive recording an inhibition zone (IZ) diameter of 0-8 mm, partially active (9-11 mm IZ) at 100-200 mg, and active (11-15 mm IZ) at 300 mg/ ml. At 25-100 mg/ ml, the chloroform extract expressed partial activity against all the tested microorganisms recording an IZ of 11-12 mm, active (13-15 mm IZ) against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*, and very active (20 mm IZ) against *C. albicans* at 300 mg/ ml. Finally, at 25-100 mg/ ml; the aqueous extract had no activity (0-8 mm IZ) against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*, but was partially active against all the tested microorganisms at 200-300 mg/ ml; recording IZ of 9-11 mm. Findings of this study revealed that *A. javanica* plant extracts could be used as potent antimicrobial agents against the harmful microorganisms in the food and pharmaceutical industries.

Keywords: *Aerva javanica*, Phytochemical, Chemical composition, Antimicrobial potential

1. Introduction

Medicinal plants are chemical resource for the pharmaceutical industry, since they are key sources of traditional medicines; modern medications, nutraceuticals and pharmaceutical intermediates. Accordingly, they have attracted the attention due to their wide range of applications (Alshehri, 2020). In the developed countries, traditional medicine; also known as complementary and alternative medicine, is of rapidly growing interest in the health care systems. Moreover, traditional medicine attracted much attention from wide range of health system stakeholders, due to its accessibility and affordability for the poor people, in addition to the risk of using the chemical drugs in the allopathic medicine (Rashid *et al*., 2018). Medicinal plants are still being employed in traditional medicinal practices in Saudi Arabia, where they continue to play an important role in the human health, and are used in treatment of the ailments among the tribal; local people and medicinal healers (Suleiman, 2019).

Aerva javanica (family *Amaranthaceae*) is an erect, heavily branched perennial herb native to Africa and is found all over the world. Different parts of this plant are used in folk medicine for treatment of a variety of ailments, including chest pain and diarrhea, and are used as diuretic and demulcent agents (Arbab *et al*., 2016). *A. javanica* has antibacterial, antifungal, antiulcer, and smooth muscle relaxant properties (Nawaz *et al*., 2015).

A previous research conducted by Chawla *et al*., (2012) reported that polyphenols, terpenoids, flavonoids and alkaloids were discovered in *A. javanica* after a phytochemical investigation. According to Karthishwaran *et al*., (2018), the entire *A. javanica* plant is used for treating chest pain, ascaris and bloody diarrhea. Bacterial resistance to conventional medications is becoming a major public health issue in treatment of the infectious diseases. Antimicrobial medications are often employed against infectious diseases and MDR disorders for treatment of the bacterial diseases (Behera and Ghosh, 2018).

The Kingdom of Saudi Arabia is one of the richest biodiversity countries in the Arabian Peninsula (Suleiman, 2019). The southwestern region of Saudi Arabia has the highest plant species diversities and the most endemic ones (Ali *et al*., 2017), where the majority of the medicinal plants are found in the mountain chains that have been heavily populated with human settlements since the ancient times (Tounekti *et al*., 2019). The region of Al-Baha is located in the Saudi Arabia's southwest region and contains a diverse range of geographical regions that serve as vast repositories for the medicinal plants, which are becoming increasingly popular in the traditional medical treatments (Alshehri, 2020).

The objectives of this study were to determine the chemical composition, and *in vitro* antimicrobial activities of several extracts of *A. javanica*; collected from Shada Mountain in Al-Baha area, Saudi Arabia.

2. Materials and methods

2.1. Collection of plant samples

Fresh leaves of *A. javanica* were collected from Shada Mountain, Al-Baha, Kingdom of Saudi Arabia; during the period of March-April 2021, and were used to prepare the ethanol, chloroform and aqueous extracts. The plant was taxonomically identified and authenticated in Department of Biology, Faculty of Science and Arts, Al-Mikhwah, Al-Baha University, and then a voucher specimen was deposited in the department for further use. The leaves were washed with fresh water to remove the soil and dust particles, cut into small pieces, dried under shade for 2 weeks, macerated into a fine powder using an electric grinder (Alsaif-Elec, E03408, China), and then stored frozen for further use (Sukhdev *et al*., 2008).

2.2. Preparation of *A. javanica* extracts

2.2.1. Ethanol and chloroform extraction

Extraction from *A. javanica* was carried out using ethanol and chloroform solvents according to Sukhdev *et al*., (2008). Approximately 200 g of a finely powdered plant leaf sample was soaked individually with 100 ml of chloroform and ethanol for 3 d, filtered daily, and then the solvents were evaporated under reduced pressure using a rotary evaporator (RE-501 5 l, Henan Lanphan Industry Co., Ltd., China), stored at 4°C for further use.

2.2.2. Aqueous extraction

About 200 g of a finely powdered plant leaf sample was soaked in 100 ml of boiling dist. water with continuous stirring for 4 h. After cooling, the aqueous extract was filtered using a Whatman filter paper, and then the filtrate was kept in the refrigerator till being used (Sukhdev *et al*., 2008).

2.3. Determination of the plant extract yield

The sample extract was allowed to air dry in an evaporating dish till complete dryness, and then the yield was calculated as follows, according to Sukhdev *et al*., (2008)

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant sample}} \times 100$$

2.4. Preliminary phytochemical screening

Phytochemical analysis of the *A. javanica* extracts was carried out to detect the presence of tannins, saponins; alkaloids, flavonoids, coumarins and steroids, according to the methods conducted by Njoku and Obi, (2009); Alloui *et al*., (2020).

2.5. Analysis of the ethanol, chloroform and aqueous plant extracts by GC/MS

Various components of the ethanol, chloroform and aqueous extracts were identified and quantified using a Gas chromatography/mass spectrophotometer (GC/MS-QP2010-Ultra, Shimadzu Company, Japan). An aliquot of 0.1 µl of the sample extract was dissolved in ethanol and injected in a split mode (a ratio of 1:10) into the capillary column (Rtx-5ms-30m×0.25mm×0.25 µm). The sample was injected using helium as the carrier gas that passed with a flow rate of 1.61 ml/ min. The oven temperature program started from 60°C with the rate of 10°C/ min. to 300°C/ min. as the final temperature; with 6 min. as a holding time. The injection port used temperature was 300°C; the manipulated ion source temperature was 200°C, whereas 250°C was the interface temperature. The sample was analyzed using the scan mode in the range of mass number/ charge number (m/z) of 40-500 charges to ratio, and the total run time was 30 min. Chemical constituents of the 3 extracts were identified by comparing their retention times and mass spectra (MS) to the reference spectra in the mass spectrometry data center of the National Institute of Standards and Technology (NIST), in reference to Boudjema *et al*., (2018).

2.6. Determination of the antimicrobial efficacies of the extracts

2.6.1. Microbial cultures

The bacterial strains of *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), in addition to the fungal strain *Candida albicans* (ATCC 7596); were used for detecting the antimicrobial activities of the 3 extracts. All these microbial strains were provided by the National Research Centre, Khartoum, Sudan.

2.6.2. Preparation of the bacterial inocula

Aliquots of 1 ml of 24-h broth cultures of the tested bacterial strains were aseptically inoculated onto Nutrient agar slopes, and then incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 10 ml sterile normal saline, to produce a homogenous suspension containing about 10^8 cfu/ ml (equivalent to McFarland standard 0.5), which was stored at 4°C till being used. The average number of viable bacterial cells/ ml of the stock

suspension were determined using the surface viable counting technique of Altug *et al*., (2010). Serial dilutions of the stock suspensions were prepared in a sterile normal saline solution. About 0.02 ml of the appropriate dilution of each bacterial suspension was inoculated onto the surface of solidified NA plates, which were then incubated at 37°C for 24 h. After incubation, the number of developed bacterial colonies in each plate was counted, and then the viable count of each stock suspension was expressed as cfu/ ml suspension.

2.6.3. Preparation of the fungal suspension

The culture of the fungal strain was maintained on Sabouraud dextrose agar (SDA) slants, and then incubated at 25°C for 4 d. After incubation, the growing fungal culture was harvested through washing with sterile normal saline solution, and then its suspension was stored at 4°C.

2.6.4. The disc diffusion assay

The disc diffusion method was used to test the antibacterial and antifungal potencies of the plant extracts on Mueller Hinton agar (MHA) and SDA media; respectively, according to the previous methods conducted by Alves *et al*., (2000); Mukhtar and Ghori, (2012); CLSI. (2017). The bacterial and fungal suspensions were diluted using a sterile normal saline solution to 10^8 cfu/ ml (Turbidity= McFarland standard 0.5). An aliquot of 100 µl of the bacterial and fungal suspensions were spread uniformly on the surface of MHA and SDA plates; respectively, using a sterile glass spreader. Sterile filter paper discs (Whatman no. 1, of 6 mm in diameter) were soaked with 20 µl of each plant extract, allowed to dry, and then were placed individually on the surfaces of the MHA and SDA. The inoculated plates were incubated at 37°C for 24 h and at 30°C for 96 h, for the bacterial and fungal strains; respectively. The assays were conducted in triplicates. After incubation, the diameter (mm) of the inhibition zone (IZ) was measured using a calibrated ruler. Results of the antimicrobial activities were expressed based on the diameters of the IZ as follows: < 9 mm (resistant strain); 9-12 mm (partially resistant strain); 13-18 mm (sensitive strain), and > 18 mm (very sensitive strain).

2.7. Statistical analysis

Statistical analysis was carried out using Statistical Analysis Systems (SAS. 2002). The general linear model (GLM) procedure was used. Duncan's multiple range test was conducted for mean separation between treatments ($p \leq 0.05$). The data were reported as mean ± standard deviation of the triplicate determinations.

3. Results

3.1. Yield of *A. javanica* extract

The average yield (%) of *A. javanica* extracts is shown in Table (1). The yield of the aqueous extract (11.01± 0.004 %) was statistically ($p< 0.001$) higher than the yields of the ethanol (7.48± 0.004 %) and chloroform (5.60± 0.04 %) extracts.

3.2. Phytochemical screening of the *A. javanica* extracts

Phytochemical screening of the *A. javanica* extracts revealed the presence of coumarins and steroids in the ethanol extract, while saponins; coumarins, tannins, flavonoids and steroids were detected in the chloroform and aqueous extracts (Table 2).

3.3. Chemical composition of the *A. javanica* leaf extracts

Tables (3-5) list the bioactive compounds detected in the ethanol, chloroform and aqueous leaf extracts of *A. javanica* using the GC/MS. About 38 compounds were identified in the ethanol extract with the maximum peak area being of benzyldiethyl (2,6-xylylcarbamoylmethyl) ammonium benzoate (17.25 %) with a retention time of 21.742 min., while the minimum peak area of this extract was of 2-[(trimethylsilyl)oxy]- (0.09%) with a recorded retention time of 3.960 min. according to the results

presented in Table (3). The other prominent components included; 1-nonadecene (10.36 %), 9-octadecenoic acid methyl ester (E) (10.32 %), 1-heptadecene (6.67 %), vitamin E (6.11 %), hexadecanoic acid methyl ester (5.61 %), l-norvaline, N-(2- (4.54 %), phytol (3.95 %), methyl stearate (3.42 %) and 1-tetradecene (3.04 %). Table (4) revealed the presence of 41 bioactive compounds in the chloroform extract, with phytol acetate (9.45 %) having the highest peak area with a retention time of 14.885 min., and decane (0.10 %) having the lowest peak area with a retention time of 3.959 min. The remaining important chemicals recorded were 1-nonadecene (8.12 %), phenol, 2,4-bis (1,1-dimethylethyl)- (8.10 %), tetracontane (6.94 %), phytol (6.70 %), squalene (6.70 %), 1-heptadecene (3.95 %), vitamin E (3.89 %), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (3.81 %), 9-octadecenoic acid (Z) methyl ester (3.63 %) and floxuridine (3.39 %). Finally, in the aqueous extract, a total of 27 compounds were identified; with hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (17.94 %) having the highest peak area with a retention time of 21.456 min. and oxirane, [(hexadecyloxy) methyl]- (0.44 %) having the lowest peak area with a retention time of 13.055 min. The remaining main components were; 9-octadecenoic acid (Z) methyl ester (13.79 %), methyl 10-trans,12-cis-octadecadienoate (11.51 %), hexadecanoic acid methyl ester (10.39 %), octadecanoic acid, 2,3-dihydroxypropyl (6.59 %), 1-ethylsulfanylmethyl-2,8,9-trioxa-5-aza- (4.17 %), 1-heptadecene (3.77 %), 1-nonadecene (3.52 %), methyl stearate (3.52 %), and 1,3,5-trisilacyclohexane (3.01 %), as demonstrated in Table (5). The major compounds detected in the 3 extracts were benzyldiethyl (2,6-xylylcarbamoylmethyl) ammonium benzoate in the ethanol extract, phytol acetate in the chloroform extract, whereas hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester were recorded in the aqueous extract.

Table 1: Yields (%) of *A. javanica* leaves extracts

Types of extracts	Weight of the plant powder (g)	Weight of the crude extract (g)	Yield (%)
Ethanol extract	200	14.96 ± 0.01^{b}	7.48 ± 0.004^{b}
Chloroform extract	200	11.20 ± 0.08^{c}	5.60 ± 0.04^{c}
Aqueous extract	200	22.01 ± 0.01^{a}	11.01 ± 0.004^{a}

Where; Means within the same column bearing different superscript letters are significantly different at $p< 0.05$. N = 3, (±): Standard deviation (SD)

Table 2: Phytochemical screening of *A. javanica* leaves extracts

Phytochemical components	Types of extracts		
	Ethanol	**Chloroform**	**Aqueous**
Saponin	-	+	+
Coumarin	+	+	+
Alkaloids	-	-	+
Tannins	-	+	-
Flavonoids	-	+	-
Steroids	+	+	-

Where; + = Present, - = Absent

Table 3: Chemical composition of the *A. javanica* ethanol leaf extract

S.N.	RT (min.)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	3.407	Butane, 1,1,3-trimethoxy-	$C_7H_{16}O_3$	148	0.29
2	3.529	Benzoic acid, 2-formyl-4,6-dimethoxy-, 8,8-	$C_{20}H_{30}O_7$	382	0.17
3	3.960	Ethanol, 2-[(trimethylsilyl)oxy]-	$C_5H_{14}O_2Si$	134	0.09
4	9.305	1-Tetradecene	$C_{14}H_{28}$	196	1.66
5	9.395	1-Tetradecene	$C_{14}H_{28}$	196	3.04
6	10.192	Cyclohexane, octyl-	$C_{14}H_{28}$	196	0.80
7	11.920	5-Undecene, 3-methyl-, (E)-	$C_{12}H_{24}$	168	2.33
8	12.002	1-Heptadecene	$C_{17}H_{34}$	238	6.65
9	12.078	Hexadecane	$C_{16}H_{34}$	226	0.73
10	14.290	Tridecane, 3-methylene-	$C_{14}H_{28}$	196	0.94
11	14.362	1-Nonadecene	$C_{19}H_{38}$	266	6.07
12	14.435	Heneicosane	$C_{21}H_{44}$	296	0.79
13	14.888	Phytol, acetate	$C_{22}H_{42}O_2$	338	2.00
14	15.154	9-Octadecen-1-ol, (Z)-	$C_{18}H_{36}O$	268	0.45
15	15.210	Dodecylcyclohexane	$C_{18}H_{36}$	252	0.48
16	15.327	8-Octadecanone	$C_{18}H_{36}O$	268	0.69
17	15.361	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.84
18	15.852	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.61
19	16.505	1-Nonadecene	$C_{19}H_{38}$	266	4.29
20	16.550	Pentanoic acid, ethyl ester	$C_7H_{14}O_2$	130	1.12
21	17.590	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	2.29
22	17.637	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	10.32
23	17.781	Phytol	$C_{20}H_{40}O$	296	3.95
24	17.865	Methyl stearate	$C_{19}H_{38}O_2$	298	3.42
25	17.984	l-Norvaline, N-(2-methoxyethoxycarbonyl)-,	$C_{21}H_{41}NO_5$	387	4.54
26	18.460	n-Tetracosanol-1	$C_{24}H_{50}O$	354	1.41
27	20.258	1-Nonadecene	$C_{19}H_{38}$	266	1.21
28	21.026	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	1.40
29	21.169	2-Ethylbutyric acid, eicosyl ester	$C_{26}H_{52}O_2$	396	1.72
30	21.243	13-Docosenoic acid, methyl ester, (Z)-	$C_{23}H_{44}O_2$	352	0.67
31	21.432	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	$C_{19}H_{38}O_4$	330	1.77
32	21.680	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	0.67
33	21.742	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-a	$C_{28}H_{34}N_2O_3$	446	17.25
34	22.083	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,1	$C_{32}H_{54}O_3$	486	0.82
35	22.648	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	0.70
36	23.478	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	0.62
37	25.868	Tetracontane	$C_{40}H_{82}$	562	1.08
38	26.624	Vitamin E	$C_{29}H_{50}O_2$	430	6.11
					100.00

Where; RT: Retention time

Table 4: Chemical composition of the *A. javanica* chloroform leaf extract

S.N.	RT (min.)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	3.868	1-Decene	$C_{10}H_{20}$	140	0.48
2	3.959	Decane	$C_{10}H_{22}$	142	0.10
3	6.566	1-Dodecene	$C_{12}H_{24}$	168	1.58
4	6.671	Dodecane	$C_{12}H_{26}$	170	0.47
5	9.391	1-Tetradecene	$C_{14}H_{28}$	196	3.21
6	11.216	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	4.89
7	11.999	1-Heptadecene	$C_{17}H_{34}$	238	3.95
8	14.360	1-Nonadecene	$C_{19}H_{38}$	266	4.01
9	14.885	Phytol, acetate	$C_{22}H_{42}O_2$	338	9.45
10	15.157	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.29
11	15.359	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.81
12	15.845	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.58
13	16.500	1-Nonadecene	$C_{19}H_{38}$	266	4.11
14	16.545	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.34
15	17.585	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	1.47
16	17.632	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	3.63
17	17.775	Phytol	$C_{20}H_{40}O$	296	6.70
18	17.979	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-s	$C_9H_{19}NO_3SSi$	249	1.60
19	18.454	n-Tetracosanol-1	$C_{24}H_{50}O$	354	2.54
20	18.993	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	0.20
21	19.493	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-e	$C_{35}H_{68}O_5$	568	0.80
22	20.066	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1	$C_{28}H_{42}O_2$	419	2.05
23	20.250	1-Heptacosanol	$C_{27}H_{56}O$	396	2.88
24	21.020	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	1.82
25	21.165	Floxuridine	$C_9H_{11}FN_2O_5$	246	3.39
26	21.427	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	$C_{19}H_{38}O_4$	330	0.44
27	21.910	Heneicosyl trifluoroacetate	$C_{23}H_{43}F_3O_2$	408	1.59
28	22.077	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,1	$C_{32}H_{54}O_3$	486	1.45
29	22.716	Tetracontane	$C_{40}H_{82}$	562	1.16
30	23.063	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	0.73
31	23.470	Hexatriacontane	$C_{36}H_{74}$	506	1.82
32	23.581	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,1	$C_{32}H_{54}O_3$	486	0.36
33	23.766	Squalene	$C_{30}H_{50}$	410	6.70
34	24.202	Tetracontane	$C_{40}H_{82}$	562	3.72
35	24.977	Pentatriacontane	$C_{35}H_{72}$	492	1.17
36	25.860	Tetracontane	$C_{40}H_{82}$	562	3.22
37	26.423	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	678	1.47
38	26.610	Vitamin E	$C_{29}H_{50}O_2$	430	3.86
39	26.850	1-Dodecanol, 2-octyl-	$C_{20}H4_{2O}$	298	0.38
40	28.354	Stigmasterol	$C_{29}H_{48}O$	412	2.17
41	29.154	.gamma.-Sitosterol	$C_{29}H_{50}O$	414	2.41
					100.00

Where; RT: Retention time

Table 5: Chemical composition of the *A. javanica* aqueous leaf extract

S.N.	RT (min.)	Name	Molecular formula	Molecular weight	Peak area (%)
1	6.558	1-Tridecene	$C_{13}H_{26}$	182	0.66
2	6.593	Cyclohexane, 1,5-diethyl-2,3-dimethyl-	$C_{12}H_{24}$	168	1.44
3	7.281	Cyclohexane, hexyl-	$C_{12}H_{24}$	168	1.24
4	9.350	1-Hexadecanol	$C_{16}H_{34}O$	242	1.11
5	9.402	1-Tetradecene	$C_{14}H_{28}$	296	1.75
6	11.960	1-Pentadecene	$C_{15}H_{30}$	210	1.43
7	12.014	1-Heptadecene	$C_{17}H_{34}$	238	3.77
8	13.055	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	298	0.44
9	14.379	1-Nonadecene	$C_{19}H_{38}$	266	3.52
10	15.868	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	10.39
11	16.524	1-Nonadecene	$C_{19}H_{38}$	266	1.69
12	17.415	Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis-	$C_9H_{20}O_2Si$	188	0.85
13	17.612	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	11.51
14	17.658	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	13.79
15	17.715	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.89
16	17.886	Methyl stearate	$C_{19}H_{38}O_2$	298	3.52
17	18.006	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-s	$C_9H_{19}NO_3SSi$	249	4.17
18	19.520	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	1.50
19	21.050	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	3.01
20	21.195	2-Ethylbutyric acid, eicosyl ester	$C_{26}H_{52}O_2$	396	1.23
21	21.266	13-Docosenoic acid, methyl ester, (Z)-	$C_{23}H_{44}O_2$	352	1.25
22	21.456	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	$C_{19}H_{38}O_4$	330	17.94
23	21.550	Hexadecanoic acid, 2-hydroxy-1-(hydroxyme	$C_{19}H_{38}O_4$	330	1.40
24	21.771	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-a	$C_{28}H_{34}N_2O_3$	446	2.12
25	22.674	Formamide, N-{4-[2-(1,1-dimethylethyl)-5-o	$C_{12}H_{21}NO_4$	243	1.48
26	23.092	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	385	6.59
27	25.905	Hexatriacontane	$C_{36}H_{74}$	506	1.29
					100.00

Where; RT: Retention time

3.4. Antimicrobial potentials of leaf extracts of *A. javanica*

The antibacterial activities of the ethanol, chloroform and aqueous extracts of *A. javanica* leaves against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*, as well as the antifungal activity against *C. albicans*, were investigated. Table (6) showed that as the concentration of the ethanol extract increased from 25 mg/ ml to 300 mg/ ml, the activity of the extract increased. The ethanol extract was inactive (0-8 mm IZ) against *B. cereus*, *C. albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* at a dosage of 25 mg/ ml, partially active (11-12 mm IZ) against *B. cereus*, *P. aeruginosa*, and *S. aureus* at 300 mg/ ml, and active (13.0± 0.84 mm, 15.0± 0.75 IZ) against *E. coli* and *C. albicans*; respectively, at 300 mg/ ml. The chloroform extract was partially active (11-12 mm IZ) at a dosage of 25-100 mg/ ml, and active (13-16 mm IZ) at a dosage of 200-300 mg/ ml against all the microorganisms tested, with the exception of *C. albicans*, which was very sensitive (20.0± 1.00 mm IZ) at the dosage of 300 mg/ ml (Table 7). The activity of the aqueous extract increased as its

concentration rose (Table 8). The extract was inactive (0-8 mm IZ) against *B. cereus*, *E. coli* and *S. aureus* at dosages ranging from 25- 100 mg/ ml, and partially active (9-11 mm IZ) at 200-300 mg/ ml. At 25 mg/ ml, the aqueous extract was inactive (8 mm IZ) against *C. albicans* and *P. aeruginosa*, but the extract was partially active (9-11 mm IZ) against *C. albicans* and *P. aeruginosa* at 50-300 mg/ ml (Table 8). Statistically, the antibacterial activity of the chloroform extract was found to be the highest ($p< 0.001$), followed by the ethanol and the aqueous extracts (Table 9).

Table 6: Inhibition zones (mm) of different concentrations of *A. javanica* leaf ethanol extract against the tested microorganisms

Microorganisms	Extract concentration (mg/ ml)				
	25	50	100	200	300
B. cereus	8.0± 0.98^{c}	8.0± 1.03^{c}	9.0± 1.00^{bc}	10.0± 0.41^{b}	11.0± 0.52^{a}
C. albicans	0.0± 0.0^{e}	8.0± 0.63^{d}	9.0± 0.65^{c}	11.0± 0.64^{b}	15.0± 0.75^{a}
E. coli	0.0± 0.0^{e}	8.0± 0.55^{d}	9.0± 0.52^{c}	10.0± 0.63^{b}	13.0± 0.84^{a}
P. aeruginosa	8.0± 0.63^{c}	8.0± 0.63^{c}	9.0± 0.41^{bc}	10.0± 1.26^{b}	12.0± 0.80^{a}
S. aureus	8.0± 0.75^{c}	8.0± 0.98^{c}	9.0± 0.60^{bc}	10.0± 0.84^{b}	12.0± 0.60^{a}

Where; Means within the same column bearing different superscript letters are significantly different at $p< 0.05$. N = 3, (±): Standard deviation (SD)

Table 7: Inhibition zones (mm) of different concentrations of *A. javanica* leaf chloroform extract against the microorganisms tested

Microorganisms	Extract concentration (mg/ ml)				
	25	50	100	200	300
B. cereus	11.0± 0.84^{d}	12.0± 0.65^{c}	12.0± 1.03^{c}	13.0± 0.63^{b}	15.0± 1.03^{a}
C. albicans	12.0± 0.64^{c}	12.0± 0.98^{c}	12.0± 0.85^{c}	16.0± 1.26^{b}	20.0± 1.00^{a}
E. coli	11.0± 0.55^{d}	11.0± 0.63^{d}	12.0± 0.70^{c}	13.0± 0.54^{b}	15.0± 0.76^{a}
P. aeruginosa	11.0± 0.63^{d}	12.0± 0.64^{c}	12.0± 0.75^{c}	13.0± 0.75^{b}	15.0± 1.04^{a}
S. aureus	12.0± 0.99^{b}	12.0± 0.85^{b}	12.0± 0.84^{b}	14.0± 0.82^{a}	14.0± 0.90^{a}

Where; Means within the same column bearing different superscript letters are significantly different at $p< 0.05$. N = 3, (±): Standard deviation (SD)

Table 8: Inhibition zones diameters (mm) of different concentrations of *A. javanica* aqueous leaf extract against the tested microorganisms

Microorganisms	Extract concentration (mg/ ml)				
	25	50	100	200	300
B. cereus	0.0± 0.00^{c}	8.0± 0.55^{b}	8.0± 0.80^{b}	10.0± 0.85^{a}	10.0± 0.66^{a}
C. albicans	8.0± 0.80^{b}	11.0± 0.75^{a}	11.0± 0.60^{a}	11.0± 0.75^{a}	11.0± 0.52a
E. coli	0.0± 0.00^{c}	8.0± 0.89^{b}	8.0± 0.70^{b}	10.0± 1.03^{a}	10.0± 1.05^{a}
P. aeruginosa	8.0± 0.75^{d}	9.0± 0.63c	9.0± 1.00^{c}	10.0± 1.01^{b}	11.0± 0.90^{a}
S. aureus	0.0± 0.00^{c}	0.0± 0.00^{c}	0.0± 0.00^{c}	9.0± 0.82^{b}	11.0± 0.75^{a}

Where; Means within the same column bearing different superscript letters are significantly different at $p< 0.05$. N = 3, (±): Standard deviation (SD)

Table 9: Antimicrobial potential of *A. javanica* leaf extracts

Microorganisms	Diameters of inhibition zones (mm)		
	Ethanol extract	**Chloroform extract**	**Aqueous extract**
B. cereus	9.20± 1.21^{b}	12.80± 1.01^{a}	7.20± 3.84^{c}
C. albicans	8.60± 5.09^{b}	14.40± 2.75^{a}	10.40± 1.24^{b}
E. coli	8.00± 4.49^{b}	12.42± 1.66^{a}	7.20± 3.84^{b}
P. aeruginosa	9.40± 1.55^{b}	12.60± 1.66^{a}	9.40± 1.06^{b}
S. aureus	9.40± 1.55^{b}	12.80± 0.41^{a}	4.00± 5.11^{c}

Where; Means within the same column bearing different superscript letters are significantly different at $p< 0.05$. N = 3, (±): Standard deviation (SD)

4. Discussion

Herbal plants have been studied in depth to find novel pharmaceutical components and to develop new medications, and these medicinal plants could be used as new and effective antimicrobial agents (Al-Shehri and Moustafa, 2019). The medicinal plants in Al-Baha region have pharmacological and antibacterial properties. The Amaranthaceae family, which includes *A. javanica*, is a common plant family in Al-Baha area, with around 180 genera and 2500 species (Alshehri, 2020). Currently, the aqueous extract produced a higher yield than the ethanol and chloroform extracts. In this study, the yield of the ethanol extract was in agreement with the findings of Suleiman, (2019) that the ethanolic extract of aerial portions of *A. javanica* was 6.7 %, and the results of Movaliya and Zaveri's, (2020), who recorded that the yield of ethanolic root extract of *A. javanica* was 6.25 %. The chloroform extract yield matches the findings of Karthishwaran *et al*., (2018), who found a yield of 5.12 % for *A. javanica* air dried powdered leaves extracted with chloroform.

A previous study conducted by Kumar *et al*., (2013) reported that the plant bioactive compounds are non-nutritive molecules produced by the plants as self-defense, to protect themselves from pests, pathogens and the various environmental stresses. The qualitative phytochemical screening of the ethanol, chloroform and aqueous extracts of *A. javanica* leaves showed the presence of saponins, coumarins, alkaloids, tannins, flavonoids and steroids. Similar findings were reported by Arbab *et al*., (2016), who recorded the presence of alkaloids, flavonoids, tannins, steroids and saponins in the ethanolic extract of *A. javanica* leaves, and the study of Suleiman, (2019), who revealed the existence of alkaloids, saponins, triterpens, tannins and flavonoids in the ethanol extract of *A. javanica* aerial parts. Furthermore, the previous studies conducted by Nawaz *et al*., (2015); Ranjan and Deokule, (2013), detected the presence of flavonoids; saponins and tannins in the ethanol and aqueous extracts of *A. javanica* leaves, and tannins; saponins, tannic acid, flavonoids, steroids and alkaloids in *A. javanica* roots extracted using ethanol and water, respectively. The presence of terpenes and anthraquinones in the whole *A. javanica* plant extracted with ethanol and chloroform was reported by Abbas *et al*., (2015), whereas the existence of glycosides and phenols in the chloroform extract of *A. javanica* leaf was reported by Srinivas and Reddy, (2012); however, these phytochemicals were not detected in the current study.

During this study, GC/MS analysis was used to verify the chemical components of the ethanol, chloroform and aqueous extracts of *A. javanica* leaves. In the ethanol, chloroform and aqueous extracts, a total of 38, 41, and 27 bioactive compounds were detected, respectively. The main compounds recorded were; benzyldiethyl (2,6-xylylcarbamoylmethyl) ammonium benzoate, 1-nonadecene and 9-octadecenoic acid methyl ester (E) in the ethanol extract; phytol acetate, 1-nonadecene, phenol, 2,4-bis (1,1-dimethylethyl)- (8.10 %), tetracontane, phytol and squalene in the chloroform extract; and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9-octadecenoic acid (Z) methyl ester, methyl 10-trans,12-cis-octadecadienoate and hexadecanoic acid methyl ester in the aqueous extract. These results are not in line with the previous findings of Karthishwaran *et al*., (2018), who identified 2-chlorallyl diethyldithiocarbamate (CDEC), carbaril, bis(2-ethylhexyl) phthalate, quinoline, 4H cyclopenta[def] phenanthrene, 2-[bis(2-chloroethylamino)]-tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide, phenobarbital, 1H-indole, 2-methyl-, 2,3,7,8-tetrachlorodibenzo-p-dioxin, disulfide and diphenyl; as the major bioactive compounds in n-hexane, chloroform, ethyl acetate, acetone and methanol extracts of the aerial parts of *A. javanica*.

The emergence of MDR bacteria is attributed to the widespread use of synthetic drugs (Katsumi *et al*., 2005). The pharmaceutical companies are turning to

plants to produce effective antimicrobial treatments and tackle the problem of resistance-breaking strains of microorganisms, evolved due to the increased use of antibiotics (Mufti *et al*., 2012). In this study, with increasing their concentrations; the activities of the ethanol, chloroform and aqueous extracts increased against all the tested microorganisms. The ethanol extract was inactive against the tested microorganisms at the doses of 25-100 mg/ ml, partially active at 200 mg/ ml and active at 300 mg/ml, whereas the chloroform extract was partially active at the doses of 25-100 mg/ ml and active at doses of 200-300 mg/ ml, except for *C. albicans*; where the extract was very active at the dose of 300 mg/ ml. At the doses of 25-100 mg/ ml, the aqueous extract was inactive against the microorganisms, with the exception of *C. albicans* and *P. aeruginosa*; where the extract was partially active against them at 50-100 mg/ ml. The aqueous extract was partially active against the tested microorganisms at the doses of 200-300 mg/ ml. The current findings of activity of the chloroform extract are consistent with the previous results of Mufti *et al*., (2012), who found that the chloroform and aqueous extracts of whole plant of *A. javanica* were effective against *E. coli*, *P. aeruginosa* and *S. aureus*. Moreover, a previous study of Al-Shehri and Moustafa, (2019) reported that the chloroform extract of *A. javanica* leaves had a potent antibacterial potency against *S. aureus* and *P. aeruginosa*. In this study, results of the ethanol extract activity against *S. aureus* are contradictory to those of Anand *et al*., (2014), who reported that the alcoholic extracts of the *A. javanica* plant had the highest activity against S. *aureus*. Results of the antifungal activity of the aqueous extract against *C. albicans* differ from those of Vidhya and Udayakumar, (2017), who reported the absence antifungal potential of *A. lanata* aqueous leaf extract against *C. parasilosi*, and differ from results of the study conducted by Suleiman, (2019), who reported that the ethanol extract of *A. javanica* aerial parts had no activity against *E. coli*, *S. aureus* and *C. albicans*. These currently recorded antibacterial and antifungal activities of the 3 extracts could be attributed to the presence of the various bioactive compounds in these extracts, qualitatively identified using the GC/MS assay.

Conclusion

The medicinal plant *A. javanica* is extensively dispersed in Al-Baha area; however, it was not studied to evaluate its antimicrobial efficacy against the various microorganisms, in order to be used in the sectors of food or pharmaceutical industries. Therefore this study looks into the possibility of using the extracts of this plant as antimicrobial agents. This study recorded that *A. javanica* plant contains several antioxidant phytochemicals such as alkaloids and flavonoids. Chemical analysis revealed the existence of different bioactive compounds in all extracts of this plant, which could serve as antimicrobial agents. The *A. javanica* extracts expressed significant antimicrobial potentials against the tested Gram (+) and Gram (-) bacteria, as well as *C. albicans*. Finally, special attention should be focused on the efficacies *A. javanica* extracts to be widely used on the industrial scale. This is the first time that *A. javanica* plant has been studied in Al-Baha area, Saudi Arabia.

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Conflict of interest

The author reports no potential conflict of interests.

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Ethical approval

Non-applicable.

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