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ANTIMICROBIAL ACTIVITY OF EXTRACTS AND COMPOUNDS ISOLATED FROM *CASSIA ITALICA* AERIAL PARTS

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ABSTRACT

Phytochemical investigation of *Cassia italica* Mill. (Fabaceae) aerial parts collected from Saudi Arabia gave nine compounds: *β*-sitosterol (1), (22*E*)-3-*β*-hydroxycycloart-22-en-24-one (2), uvaol (3), daucosterol (4), methyl 3,4-dihydroxybenzoate (5), emodin (6), 4-hydroxyphenyl-*O*-*β*-D-glucopyranoside (7), aloin B (8), and rutin (9). Their structures were established by extensive spectroscopic study, in addition to co-chromatography with authentic samples. The total MeOH extract (TME), different fractions, and compounds 1-9 were assessed for their antimicrobial effects using agar plate diffusion assay. The TME, EtOAc fraction, and compounds 5-9 showed significant antimicrobial activity against the tested microbial strains.

Keywords: *Cassia italic*, Sterols, Triterpenes, Phenolics, Anthraquinones, Antimicrobial.

INTRODUCTION

Cassia italic (family: Fabaceae) known as Eshrinq, is widely distributed in Saudi Arabia. In traditional medicine, *Cassia* species were used for treatment of edema, constipation, and skin infections [1]. The leaves and entire plant decoction has been used as expectorant, laxative, and purgative [2]. Moreover, it is used for intestinal and urinary tract infections as well as rheumatic disorders [2]. The EtOH extract possesses different activities: antipyretic, anti-inflammatory, anti-neoplastic, antioxidant, antiviral, hepatoprotective, analgesic, CNS depressant, antibacterial, and hypoglycemic [3-11]. Previous phytochemical studies of *C. italica* showed the presence of flavonoids [12,13], anthraquinones [11-15], sterols [12,13], triterpenes [12,13], hydrocarbons, and fatty acids [16]. The following report deals with the isolation and structure elucidation of nine compounds (1-9) from the aerial parts of *C. italica* (Fig. 1). Also, the antimicrobial activities of the TME, different fractions, and isolated compounds were assessed against eleven pathogens (Tables 1 and 2).

MATERIALS AND METHODS

General Experimental Procedures

Digital Melting Point Electrothermal (9100) apparatus was used for melting points measurement. The UV spectra measurement was performed using Perkin Elmer spectrophotometer Model 550S. Shimadzu Infrared (400) spectrophotometer was used for measuring IR spectral data. The EI- and ESIMS mass spectral measurements were carried out using Finnigan MAT TSQ 7000 and LCQ DECA mass spectrometers, respectively. NMR spectra were recorded on Bruker BioSpin 400 MHz GmbH Ultrashield spectrometer. Separations of compounds were performed on Sephadex LH-20 (Merck, 0.25-0.1 mm) and SiO_2 60 (Merck, 0.04-0.063 mm). TLC analysis was carried out using pre-coated silica gel plates (0.2 mm). MeOH:$CHCl_3$ (3:97, S1), MeOH:$CHCl_3$ (10:90, S2), and MeOH:$CHCl_3$ (15:85, S3) are the systems used for TLC analysis. TLC detection of spots was done by visualization under UV at λ_{max} 255 and 366 nm and using *p*-anisaldehyde/H_2SO_4 as spraying reagent,

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followed by heating for 1-2 min at 110 ºC.

Plant Material

The plant sample was collected from King Abdulaziz University campus in November 2011 and identified by Dr. A. A. Fayed, Faculty of Science, Assiut University, Egypt, as well as morphological features and library data base [17,18]. A voucher specimen (Registration code AXG-2011) was kept at the Natural products and Alternative Medicine Department, Faculty of Pharmacy, King Abdulaziz University, Saudi Arabia.

Extraction and Isolation

The powdered plant (500 g) was extracted with methanol (6 × 2.5 L). The MeOH extract was concentrated to give brown residue (31 g). The residue was mixed with H_2O (200 mL) and fractionated between *n*-hexane and EtOAc to obtain *n*-hexane (4.9 g), EtOAc (9.2 g), and aqueous (10.1 g) fractions, respectively. Vacuum liquid chromatography (VLC) of the *n*-hexane fraction (3.5 g) on silica gel (SiO_2) using *n*-hexane:EtOAc gradient gave three sub-fractions (A-C). SiO_2 column (60 g × 50 cm × 1 cm) of sub-fractions B (890 mg) and C (950 mg) using *n*-hexane:EtOAc gradient afforded 1 (42 mg), 2 (5.4 mg), and 3 (8.5 mg). VLC of the EtOAc fraction (7.0 g) using $CHCl_3$:MeOH gradient elution gave 6 sub-fractions (A-F). Sub-fraction B (827 mg) have been subjected to SiO_2 column chromatography using gradient elution of $CHCl_3$:MeOH to obtain 4 (62 mg). Sub-fractions C (659 mg) and D (490 mg) were chromatographed separately over SiO_2 columns using $CHCl_3$:MeOH solvent system to afford 5 (17 mg, sub-fr. C) and 6 (11 mg, sub-fr. C), and 7 (13 mg, sub-fr. D). Sub-fraction E (970 mg) was chromatographed over Sephadex LH-20 column using MeOH, followed by SiO_2 column afforded 8 (17 mg) and 9 (32 mg).

Spectral Data of the Isolated Compounds

β-Sitosterol (**1**): Colorless fine needles (42 mg), mp 139-140 °C. R_f 0.86 (S1) Co-TLC with authentic sample; IR (KBr): γ_{max} 3455 (OH), 2940, 1617 (C=C), 1454, 1385, 1062 cm^{-1}; EIMS *m*/*z*: 414 $[M]^+$.

(22*E*)-3-*β*-hydroxycycloart-22-en-24-one (**2**): Colorless needless (5.4 mg); R_f 0.69 (S1); mp 181-182 °C; IR (KBr) ν_{max} 723, 870, 1645, 1722 (C=O), 2930 (C-H), 3435 (OH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.62 (H-1A, m), 1.27 (H-1B, m), 1.59 (H-2A, m), 1.72 (H-2B, m), 3.28 (H-3, m), 1.50 (H-5, m), 1.61 (H-6, m), 1.75 (H-7A, m), 1.22 (H-7B, m), 1.28 (H-8, m), 1.97 (H-11A, m), 1.05 (H-11B, m), 2.48 (H-12A, m), 2.39 (H-12B, m), 1.36 (H-15A, m), 1.25 (H-15B, m), 1.28 (H-16, m), 1.59 (H-17, m), 0.97 (H-18, s), 0.55 (H-19A, d, *J* = 3.6 Hz), 0.32 (H-19B, d, *J* = 3.6 Hz), 2.61 (H-20, m), 1.08 (H-21, d, *J* = 6.4 Hz), 7.53 (H-22, dd, *J* = 15.8, 7.4 Hz), 7.71 (H-23, d, *J* = 15.8 Hz), 2.37 (H-25, m), 1.08 (H-26, d, *J* = 6.4 Hz), 0.86 (H-27, d, *J* = 6.4 Hz), 0.95 (H-28, s), 0.80 (H-29, s), 0.89 (H-30, s), 4.30 (3-OH, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 205.5 (C-24), 148.9 (C-23), 128.8 (C-22), 78.8 (C-3), 52.7 (C-17), 48.8 (C-14), 48.0 (C-5), 47.1 (C-8), 45.3 (C-13), 40.8 (C-20), 40.5 (C-4), 37.5 (C-12), 36.6 (C-10), 35.7 (C-15), 35.5 (C-25), 32.9 (C-1), 32.0 (C-2), 30.3 (C-19), 30.1 (C-7), 26.5 (C-16), 26.0 (C-11), 25.4 (C-28), 21.1 (C-6), 19.9 (C-9), 19.8 (C-27), 18.4 (C-30), 18.3 (C-26), 18.1 (C-21), 18.0 (C-18), 14.0 (C-29); ESIMS *m*/*z*: 441 $[M+H]^+$.

Uvaol (**3**): Colorless crystal (8.5 mg), mp 234-235 °C. R_f 0.79 (S1); ^{1}H NMR data ($CDCl_3$, 400 MHz): δ_H 5.12 (H-12, t, *J* = 3.5 Hz), 3.53 (H-28A, dd, *J* = 11.1, 5.6 Hz), 3.23 (H-3, m), 3.21 (H-28B, dd, *J* = 11.1, 5.6 Hz), 1.92 (H-11, t, *J* = 3.8 Hz), 1.81 (H-16, t, *J* = 6.1 Hz), 1.67 (H-1A, m), 1.65 (H-2, m), 1.61 (H-1B, m), 1.58 (H-21A, m), 1.56 (H-7A, m), 1.53 (H-9, m), 1.45 (H-21B, m), 1.40 (H-19, m), 1.39 (H-18, m), 1.37 (H-22, m), 1.34 (H-7, m), 1.11 (H-27, s), 1.02 (H-20, m), 1.01 (H-23, s), 1.00 (H-15, m), 0.99 (H-26, s), 0.94 (H-25, s), 0.93 (H-30, d, *J* = 6.1 Hz), 0.81 (H-29, d, *J* = 6.0 Hz), 0.79 (H-24, s), 0.74 (H-5, dt, *J* = 11.6, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 38.8 (C-1), 27.2 (C-2), 79.0 (C-3), 38.0 (C-4), 55.1 (C-5), 138.7 (C-13), 125.1 (C-12), 69.9 (C-28), 54.0 (C-18), 42.0 (C-14), 47.6 (C-9), 40.0 (C-8), 39.4 (C-19), 39.3 (C-20), 38.0 (C-17), 36.8 (C-10), 35.1 (C-22), 32.9 (C-7), 30.6 (C-21), 28.1 (C-23), 26.0 (C-15), 23.4 (C-11,16), 23.3 (C-27), 21.3 (C-30), 18.3 (C-6), 17.3 (C-26), 16.7 (C-29), 15.7 (C-25), 15.6 (C-27); ESIMS *m*/*z*: 443 $[M+H]^+$.

Daucosterol (**4**): White amorphous powder (62 mg, MeOH), R_f 0.73 (S2), Co-TLC with authentic sample.

Methyl 3,4-dihydroxybenzoate (**5**): Brown amorphous powder (17 mg), R_f 0.67 (S2); ^{1}H NMR data (DMSO-d_6, 400 MHz): δ_H 12.56 (3-OH, s), 9.88 (4-OH, s), 7.45 (H-6, dd, *J* = 7.6, 2.2 Hz), 7.42 (H-2, d, *J* = 2.2 Hz), 6.84 (H-5, d, *J* = 7.6 Hz), 3.89 (8-OCH_3, s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 121.6 (C-1), 112.7 (C-2), 147.2 (C-3), 151.1 (C-4), 115.0 (C-5), 123.5 (C-6), 167.2 (C-7), 55.5 (C-8); ESIMS *m*/*z*: 169 $[M+H]^+$.

Emodin (**6**): Brown amorphous powder (11 mg), R_f 0.64 (S2); ^{1}H NMR data (DMSO-d_6, 400 MHz): δ_H 11.97 (8-OH, s), 11.89 (1-OH, s), 11.31 (6-OH, s), 7.34 (H-4, d, *J* = 2.2 Hz), 7.02 (H-2, d, *J* = 2.2 Hz), 6.99 (H-5, d, *J* = 2.2 Hz), 6.50 (H-7, d, *J* = 2.2 Hz), 2.34 (11-CH_3, s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 161.3 (C-1), 120.4 (C-2), 148.1 (C-3), 124.0 (C-4), 134.9 (C-4a), 108.7 (C-5), 164.4 (C-6), 107.8 (C-7), 165.5 (C-8), 108.8 (C-8a), 189.5 (C-9), 113.1 (C-9a), 181.1 (C-10), 132.6 (C-10a), 21.5 (11-CH_3); ESIMS *m*/*z*: 271 $[M+H]^+$.

4-Hydroxyphenyl-*O*-*β*-D-glucopyranoside (**7**): Brown amorphous powder (13 mg), R_f 0.47 (S2); ^{1}H NMR data

(DMSO-d_6, 400 MHz): δ_H 8.99 (4-OH, s), 6.85 (H-2, 6, dd, J = 6.6, 2.2 Hz), 6.64 (H-3, 5, dd, J = 6.6, 2.2 Hz), 5.23 (H-1´, d, J = 6.5 Hz), 3.09-5.03 (sugar protons); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 152.2 (C-1), 150.4 (C-4), 117.7 (C-2,6), 115.5 (C-3,5), 101.7 (C-1`), 77.0 (C-5`), 76.6 (C-3`), 73.3 (C-2`), 69.8 (C-4`), 60.8 (C-6`); ESIMS m/z: 273 $[M+H]^+$.

Aloin B (**8**): Brown amorphous powder (17 mg), R_f 0.64 (S3); ^{1}H NMR data (DMSO-d_6, 400 MHz): δ_H 11.87 (1-OH, s), 11.78 (8-OH, s), 7.54 (H-6, d, J = 7.9 Hz), 7.06 (H-5, d, J = 7.6 Hz), 7.01 (H-4, brs), 6.84 (H-2, brs), 5.12 (H-10, d, J = 5.1 Hz), 4.55 (H-11, d, J = 8.9 Hz), 3.26 (H-1´, d, J = 9.6 Hz), 2.67- 5.41 (sugar protons); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 193.4 (C-9), 161.1 (C-1), 160.9 (C-8), 151.3 (C-3), 145.6 (C-4a), 136.1 (C-6), 118.9 (C-5), 116.2 (C-1a), 117.8 (C-7), 117.0 (C-8a), 115.4 (C-4), 112.6 (C-2), 62.4 (C-11), 44.2 (C-10), 85.2 (C-1`), 80.9 (C-5`), 79.2 (C-3`), 78.2 (C-2`), 70.1 (C-4`), 61.4 (C-6`); ESIMS m/z: 419 $[M+H]^+$.

Rutin (**9**): Yellow amorphous powder (32 mg), R_f 0.51 (S3); ^{1}H NMR data (CD_3OD, 400 MHz): δ_H 7.63 (H-2`, d, $J$ = 1.8 Hz), 7.61 (H-6`, dd, J = 6.8, 1.8 Hz), 6.87 (H-5´, d, J = 6.8 Hz), 6.38 (H-8, d, J = 1.8 Hz), 6.19 (H-6, d, J = 6.8 Hz), 5.11 (H-1``, d, J = 6.1 Hz), 4.51 (H-1```, d, J = 0.6 Hz), 3.28-3.81 (sugar protons), 1.11 (H-6```, d, J = 6.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz): δ_C 177.9 (C-4), 166.1 (C-7), 161.5 (C-5), 157.2 (C-2), 156.5 (C-9), 148.5 (C-4`), 145.2 (C-3`), 133.9 (C-3), 121.8 (C-1`), 121.7 (C-6`), 116.3 (C-5`), 115.3 (C-2`), 105.0 (C-10), 101.2 (C-1``), 100.8 (C-1```), 98.8 (C-6), 92.7 (C-8), 76.5 (C-5``), 76.0 (C-3``), 74.1 (C-2``), 71.9 (C-4```), 70.6 (C-3```), 70.4 (C-2```), 70.1 (C-4``), 68.3 (C-5```), 67.1 (C-6``), 17.3 (C-6```); ESIMS m/z: 611 $[M+H]^+$.

Antimicrobial Activity Assay:

Agar diffusion assay was used to assess antimicrobial activity of the tested compounds against eleven microorganisms. The tested organisms included Gram-positive bacteria [*Bacillus cereus* (AUMC No. B-5) and *Staphylococcus aureus* (AUMC No. B-54)], Gram-negative bacteria [*Serratia marscescens* (AUMC No. B-55), *Escherichia coli* (AUMC No. B-53), and *Pseudomonas aeurginosa* (AUMC No. B-73)], and fungi [*Geotrichium candidum* (AUMC No. 226), *Candida albicans* (AUMC No. 418), *Aspergillus flavus* (AUMC No. 1276), *Fusarium oxysporum* (AUMC No. 5119), *Trichophyton rubrum* (AUMC No. 1804), and *Scopulariopsis brevicaulis* (AUMC No. 729)]. The method was performed as previously outlined [19,20]. The TME, different fractions (250 and 500 μg/disc, DMSO), and isolated compounds (**1**-**9**) (50 and 100 μg/disc, DMSO) were used for testing antimicrobial effect. 90 mm Petri dishes, containing Mueller hinton agar (20 ml, OXOID) were used. Sterile Whatman antibiotic discs (6 mm diameter) were placed on the surface of the agar plates. The tested samples were applied onto the discs. The plates were incubated for 16-18 h at 37 °C. The zone of growth inhibition surrounding the discs was measured. Clotrimazole (50 μg/disc) and gentamycin (50 µg/disc) were used as the positive antifungal and antibacterial controls, respectively. 20 μL of DMSO loaded discs was utilized as negative controls. The inhibition zone diameter was measured by Vernier Calipers. To minimize error, all tests were repeated in triplicate.

Table 1. Antimicrobial activity results of TME and fractions

Tested strains	Inhibition zone diameter (mm/sample, Conc μg/disc)									
	n-Hexane		EtOAc		Aqueous		TME		Gent.[a]	Clot.[b]
	250	500	250	500	250	500	250	500	50	50
S. aureus	4	6	14	19	9	11	10	11	27	-
B. cereus	0	0	13	20	10	14	13	18	25	-
E. coli	0	0	10	14	7	9	8	14	23	-
P. aeurginosa	0	0	11	12	0	5	11	15	18	-
S. marscescens	0	0	6	10	1	4	3	7	28	-
C. albicans	2	5	14	20	2	4	6	11	-	22
G. candidum	0	0	11	14	0	0	12	15	-	23
F. oxysporum	0	0	13	19	0	0	14	17	-	18
A. flavus	0	0	21	26	5	11	14	19	-	32
S. brevicaulis	0	0	18	25	0	0	10	17	-	30
T. rubrum	0	0	16	23	0	0	15	21	-	30

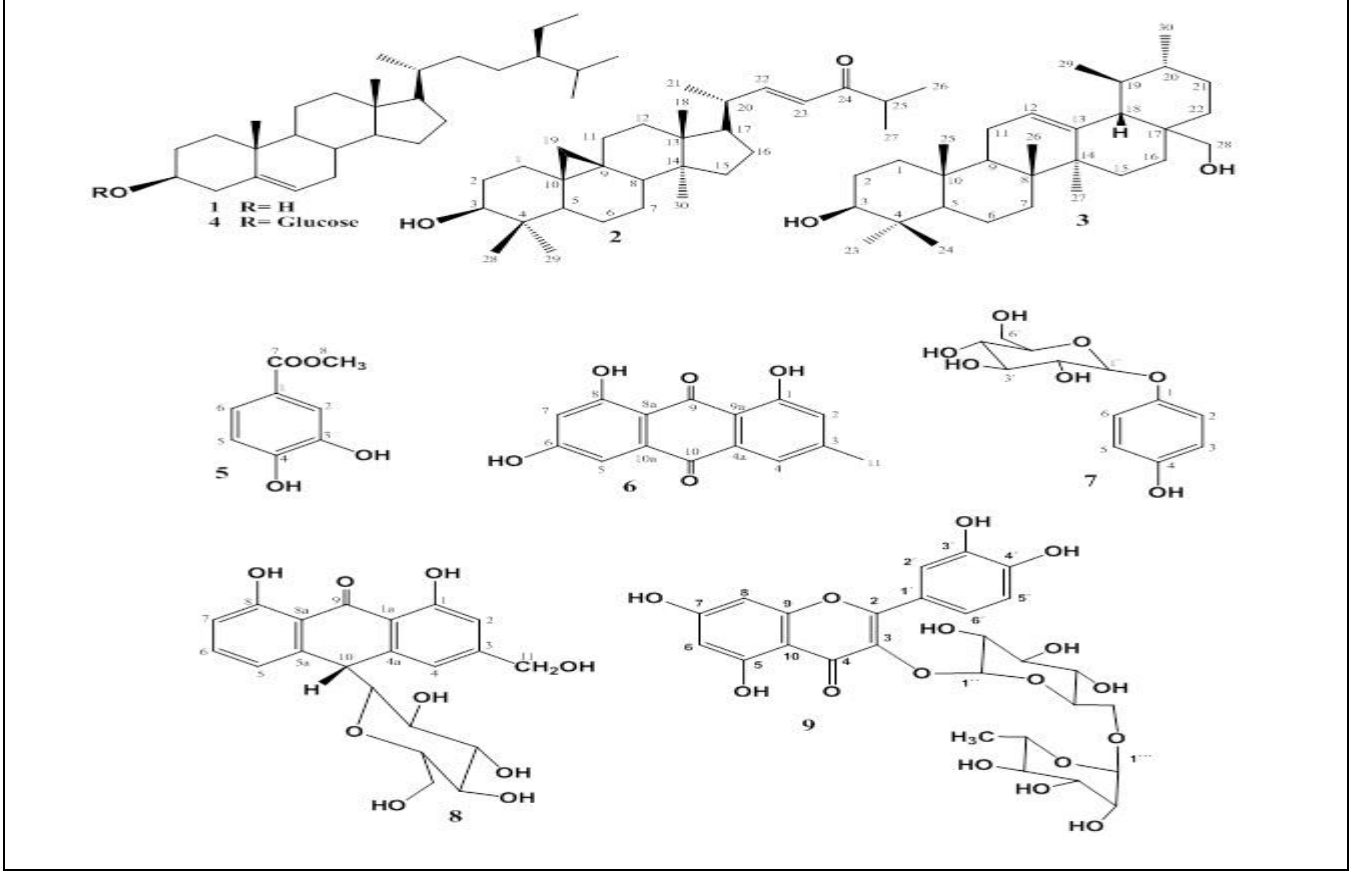
[a] Gentamycin as antibacterial standard; [b] Clotrimazole as antifungal standard.

Table 2. Antimicrobial activity results of the isolated compounds 1-9

Tested strains	Inhibition zone diameter (mm/sample)																			
	1		2		3		4		5		6		7		8		9		Gent.[a]	Clot.[b]
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	A
S. aureus	2	3	3	5	0	0	6	3	10	19	11	18	9	17	9	12	9	11	27	-
B. cereus	1	3	2	6	0	0	4	7	13	20	12	21	8	15	6	10	6	9	25	-
E. coli	0	3	0	0	9	4	9	3	10	16	9	15	9	12	11	14	9	16	23	-
P. aeurginosa	0	0	0	0	8	0	0	0	7	11	8	13	11	17	7	9	4	7	18	-
S. marscescens	0	0	0	0	0	0	0	0	12	15	10	15	10	11	13	15	0	0	28	-
C. albicans	0	0	0	5	0	5	7	5	9	14	6	11	11	16	2	3	11	16	-	22
G. candidum	0	0	0	0	0	0	0	0	13	17	11	13	0	0	4	9	5	6	-	23
F. oxysporum	4	7	0	5	0	0	3	4	10	13	7	11	0	0	7	9	0	0	-	18
A. flavus	0	2	2	4	0	0	0	0	9	10	9	15	0	0	1	0	11	14	-	32
S. brevicaulis	1	6	0	2	0	0	0	5	15	19	12	17	10	16	11	12	3	5	-	30
T. rubrum	0	0	0	0	0	0	0	0	11	16	9	21	0	0	0	0	6	7	-	30

A: 50 μg/disc; **B**: 100 μg/disc; [a] Gentamycin as antibacterial standard; [b] Clotrimazole as antifungal standard.

Fig. 1. Chemical structures of the isolated compounds 1-9



RESULTS AND DISCUSSION

The isolated compounds (Fig. 1) were identified by interpretation of their NMR and MS spectroscopic data and comparison of these data with literature as well as co-chromatography with authentic samples to be: *β*-sitosterol (1) [21], (22*E*)-3-*β*-hydroxycycloart-22-en-24-one (2) [13], uvaol (3) [22,23], daucosterol (4) [21], methyl 3,4-dihydroxybenzoate (5) [24], emodin (6) [25], 4-hydroxyphenyl-*O*-*β*-D-glucopyranoside (7) [26,27], aloin (8) [28], and rutin (9) [29].

Also, this study reports the antimicrobial activity of TME, different fractions, and isolated compounds against two Gram positive, three Gram negative bacteria, and six fungal strains. None of the *n*-hexane fraction and

compounds 1-4 showed significant activity against the tested microbial strains. The EtOAc fraction exhibited potent antimicrobial activity against the tested strains (Table 1) with inhibition zones around 26 to 10 mm (Conc. 500 μg/disc), followed by TME with varied inhibition zones around 21 to 7 mm at the same concentration. The aqueous fraction showed moderate activity towards *B. cereus*, *E. coli*, *S. aureus*, and *A. flavus* and inactive against the rest of bacterial strains. The observed antimicrobial activity of the EtOAc fraction and TME may be attributed to their high content of flavonoids, anthraquinones, and phenols [11-15]. On the other hand compounds 5-9 showed highest activity against all tested strains (Table 2) due to phenolic nature of the compounds [30]. Compound 6 showed significant activities with zone of inhibition varied from 20 to 10 (Conc. 100 μg/disc). This activity is due to the presence of *ortho*-dihydroxy phenolic hydroxyl group [30]. Compound 7 did not showed activity against *G. candidum*, *F. oxysporum*, *A. flavus*, and *T. rubrum*. Also, 8 and 9 showed no activity against *T. rubrum* and *S. marscescens*, respectively.

CONCLUSION

In conclusion, nine compounds were isolated and characterized from *C. italica*. The TME, EtOAc fraction, and compounds 5-9 displayed significant antimicrobial activity against the tested microbial strains.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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