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Research Article

NEW ANTIFUNGAL AROMATIC COMPOUNDS FROM THE SEEDS OF *RHUS CORIARIA* L.

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ABSTRACT
Phytochemical investigation of the ethanolic extract of the seeds of *Rhus coriaria* L. (Anacardiaceae) afforded three new aromatic compounds identified as 1-methoxy-4-hydroxy-methylene naphthalene (coriarianaphthyl ether), 7-methoxy-5-methyl benzen-4-al-1-oic acid (coriariaoic acid) and 1-dodecanoxy-2,8-dihydroxy-anthracene-15-oic acid (coriarianthracenyl ester) along with known phytoconstituents *n*-tetracosane, *n*-pentacosane, anise alcohol, *p*-hydroxybenzyl alcohol, methyl lawsone and 2-hydroxymethylene naphthaquinone. The structures of all the isolated compounds have been identified on the basis of spectral data analysis and chemical reactions. All the new compounds showed antifungal activity.

KEYWORDS: *Rhus coriaria* L., seeds, aromatic compounds, antifungal activity.

INTRODUCTION
Rhus coriaria L. (Anacardiaceae), commonly known as sumac, is a deciduous shrub growing up to 3 m in height in Mediterranean region, North Africa, Southern Europe, Iran and Afghanistan[1]. Sumac leaves are used as a condiment and for tanning leather; the fruits are prescribed to relieve stomach diseases, bowl complaints, fever, dermatitis, as an appetizer, diuretic and antiseptic[2-4]. Sumac is beneficial to prevent diabetes, hyperglycaemia, obesity, paralysis, colitis and diarrhoea[5,6]. The seeds are appetizer, astringent, diuretic, styptic and tonic; prescribed to treat dysentery, haemoptysis and conjunctivitis[7]. Fatty acids, flavonoids and volatile components are reported from the sumac seeds and fruits[8-14]. This paper describes isolation and characterization of three new aromatic compounds from the seeds of *R. coriaria* and their antifungal activity.

MATERIAL AND METHODS
General experimental procedures
Melting points were determined on a Perfit melting point apparatus (Ambala, India) and are uncorrected. IR spectra were recorded on KBr discs, using a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong). UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Switzerland) in methanol. ^{1}H and ^{13}C NMR spectra were scanned using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Germany), respectively, in $CDCl_3$ and TMS as an internal standard. FAB MS spectra were obtained using JEOL-JMS-DX 303 spectrometer (USA). Column chromatography was performed on silica gel (Qualigens, Mumbai, India) 60-120 mesh. TLC was run on silica gel G (Qualigens, Mumbai, India). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

Plant material

The seeds of *R. coriaria* were purchased from Khari Baoli, a local market of Delhi and authenticated by Dr. M. P. Sharma, Prof and taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen,No. PRL/JH/03/22, is deposited in the herbarium section of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and isolation of compounds

The dried drug (2 kg) was coarsely powdered, defatted with petroleum ether and then exhaustively extracted with ethanol (95%). The combined extracts were then concentrated on a water bath and dried under reduced pressure to get 110 g (5.5% yield) of dark brown mass. It was dissolved in little quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of slurry. It was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity to isolate the following compounds:

***n*-Tetracosane (1)**

Elution of the column with petroleum ether furnished buff white semisolid mass of **1**, recrystallised from $CHCl_3$-MeOH(1:1), 250 mg (0.0125% yield); R_f: 0.85 (petroleum ether); IR ν_{max} (KBr): 2911, 2850, 1210, 725, 710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (44H, brs, 22xCH_2), 0.85 (3H, t, *J*=6.1 Hz, Me-1), 0.82 (3H, t, *J*=6.0 Hz, Me-24); +ve ESI MS *m/z*: 338 $[M]^+$ ($C_{24}H_{50}$).

***n*-Pentacosane (2)**

Elution of the column with petroleum ether-$CHCl_3$ (9:1) furnished buff white semisolid mass of **2**, recrystallised from $CHCl_3$-MeOH (1:1), 300 mg (0.0150% yield); R_f: 0.75 (petroleum ether); IR ν_{max} (KBr): 2950, 2845, 1470, 1250, 1010, 725, 715 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (46H, brs, 23xCH_2), 0.85 (3H, t, *J*=6.1 Hz, Me-1), 0.82 (3H, t, *J*=6.0 Hz, Me-25); +ve ESI MS *m/z*: 352 $[M]^+$ ($C_{25}H_{52}$).

Anise alcohol (3)

Elution of the column with $CHCl_3$ afforded yellow mass of **3**, recrystallised with acetone, 275 mg (0.013% yield); R_f: $CHCl_3$-acetone (8:2); m.p.: 184-188°C; UV λ_{max} (MeOH): 256 nm (log ε 4.8); IR ν_{max} (KBr): 3448, 1655, 1543, 1219, 1010, 784 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.79 (1H, brs, D_2O exchangeable, OH), 8.31 (2H, brm, H-2, H-3), 8.16 (2H, brm, H-3, H-5), 3.74 (3H, brs, OMe), 3.43 (2H, brs, H_2-7); ^{13}C NMR (DMSO-d_6): 166.36 (C-1), 145.60 (C-4), 138.44 (C-6), 119.32 (C-2), 108.53 (C-3, C-5), 66.99 (C-7), 51.63 (OCH_3); +ve ESI MS *m/z*: 138 $[M]^+$ ($C_8H_{10}O_2$).

***p*-Hydroxybenzyl alcohol (4)**

Further elution of the column with $CHCl_3$ afforded light brown mass of **4**, recrystallised from acetone; 470 mg (0.0235% yield); R_f: 0.55 (toluene-ethyl acetate-aetic acid, 5:4.5:0.5); m.p.: 93-94°C; UV λ_{max} (MeOH): 215, 267 nm (log ε 4.1, 1.9); IR ν_{max}(KBr): 3510, 3490, 1640, 1541, 1218, 920 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.84 (1H, D_2O exchangeable, OH), 7.89 (2H, brm, H_2-2, H_2-6), 6.92 (2H, brm, H-3, H-5), 3.74 (2H, brs, H_2-7); ^{13}C NMR (DMSO-d_6): δ 168.07 (C-1), 145.78 (C-4), 138.48 (C-2), 120.86 (C-6), 109.21 (C-3, C-5), 67.39 (C-7); +ve ESI MS *m/z* (*rel. int.*): 124 $[M]^+$ ($C_7H_8O_2$) (18.1).

Methyl lawsone (5)

Elution of the column with $CHCl_3$-MeOH (99:1) furnished brown coloured powder of **5,** recrystallised from $CHCl_3$, 550 mg (0.0275% yield); R_f: 0.70 ($CHCl_3$: acetone; 3:1); m.p.: 171-73°C; UV λ_{max} (MeOH): 218, 274 nm (log ε 5.8, 2.1); IR ν_{max} (KBr): 2950, 2845, 1703, 1695, 1655, 1561, 1544, 1403, 1020, 950 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.31 (2H, m, H-6, H-9), 7.85 (2H, m, H-7, H-8), 6.94 (1H, brs, H-3), 3.74 (3H, brs, OCH_3); ^{13}C NMR (DMSO-d_6): δ 182.16, (C-1), 175.23 (C-4), 166.34 (C-2), 156.97 (C-5), 152.83 (C-10), 145.65 (C-9), 137.13 (C-6), 119.28 (C-7, C-8), 108.50 (C-3), 51.63 (OCH_3); +ve ESI MS *m/z*: 188 $[M]^+$ ($C_{11}H_8O_3$).

2-Hydroxymethylenenaphthaquinone (6)

Further elution of the column with $CHCl_3$-MeOH (99:1) afforded light brown amorphous powder of **6**, recrystallised from MeOH, 460 mg (0.0225% yield); R_f: 0.80 (toluene-ethyl acetate-aetic acid, 5:4.5:0.5); m.p.: 228-230°C; UV λ_{max} (MeOH): 217, 272 nm (log ε 4.9, 1.3); IR ν_{max} (KBr): 3510, 2950, 2840, 2342, 1700, 1695, 1653, 1551, 1370, 1050, 925, cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.10 (2H, m, H-6, H-9), 7.79 (2H, m, H-7, H-8), 6.93 (1H, brs,H-3), 4.27 (1H, brs, H_2-11a), 4.25 (1H, brs, H_2-11b); ^{13}C NMR (DMSO-d_6): δ

175.10 (C-1), 172.40 (C-4), 145.75 (C-10), 144.56 (C-9), 136.13 (C-6), 120.83 (C-7), 115.23 (C-2), 109.18 (C-3), 69.17 (C-11); +ve ESI MS *m/z*: 188 ($C_{11}H_8O_3$).

Coriarianaphthyl ether (7)

Elution of the column with $CHCl_3$-MeOH (49:1) furnished light brown amorphous mass of **7**, recrystallised from acetone, 550 mg (0.0275% yield); R_f: 0.66 (toluene-ethyl acetate-formic acid, 8.5:1:0.5); m.p.: 238-239°C; UV λ_{max} (MeOH): 217, 274 nm (log ε 5.9, 1.8); IR ν_{max} (KBr): 3450, 1640, 1541, 1390, 1219, 890 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.03 (1H, dd, *J*=8.7, 3.0 Hz, H-9), 7.96 (1H, d, *J*=9.5 Hz, H-2), 7.30 (1H, d, *J*=9.5 Hz, H-3), 7.03 (1H, dd, *J*= 9.1, 2.9 Hz, H-6), 6.79 (1H, m, H-7), 6.50 (1H, m, H-8), 3.70 (2H, brs, H_2-11), 3.41 (3H, brs, OCH_3); ^{13}C NMR (DMSO-d_6): δ 166.68 (C-1), 145.78 (C-10), 138.68 (C-5), 137.80 (C-4), 119.58 (C-2), 116.31 (C-3), 115.41 (C-7), 114.28 (C-9), 113.54 (C-6), 108.78 (C-8), 67.20 (C-11), 51.91 (OMe); +ve ESI MS *m/z*: 176 $[M]^+$ ($C_{11}H_{12}O_2$).

Coriariaoic acid (8)

Elution of the column with $CHCl_3$-MeOH (97:3) afforded brown crystals of **8**, recrystallised from MeOH-acetone (7:3), 310 mg (0.0155% yield); R_f: 0.55 ($CHCl_3$-acetone-MeOH; 7:2:1); m.p.: 226-228°C; UV λ_{max} (MeOH): 294 nm (log ε 5.3); IRν_{max}: 3144, 2925, 2860, 1710, 1690, 1541, 1219, 930 cm^{-1}; ^1H NMR ($CDCl_3$): δ 12.27 (1H, D_2O exchangeable COOH), 9.20 (1H, brs, CHO), 8.84 (1H, brs, H-3), 6.91 (1H, brs, H-6), 3.36 (3H, brs, OCH_3), 2.50 (3H, brs, CH_3-9); ^{13}C NMR (DMSO-d_6): δ 203.51 (C-8), 179.83 (C-7); 161.20 (C-2), 145.16 (C-1), 141.08 (C-4), 137.55 (C-5), 126.06 (C-3), 122.81 (C-6), 55.87 (OMe), 17.97 (C-9); +ve ESI MS (*rel. int.*) *m/z*: 194 $[M]^+$ ($C_{10}H_{10}O_4$).

Coriarianthracenyl ester (9)

Elution of the column with $CHCl_3$-MeOH (19:1) afforded brown crystals of **9**, recrystallised from MeOH, 330 mg (0.0165% yield); R_f: 0.0.60 ($CHCl_3$-acetone-MeOH; 7:2:1); m.p.: 220-222°C; UV λ_{max} (MeOH): 358 nm (log ε 3.9); IR ν_{max} (KBr): 3448, 3380, 3250, 2945, 2855, 1725, 1695, 1640, 1541, 1401, 1280, 1075, 990 cm^{-1}; ^1H NMR (DMSO-d_6): δ 13.24 (1H, brs, COOH-15), 7.99 (1H, d, *J*=8.1 Hz, H-3), 7.79 (1H, brs, H-13), 7.05 (1H, d, *J*=8.8 Hz, H-9), 6.96 (1H, d, *J*=8.1 Hz, H-4), 6.83 (1H, d, *J*=8.8 Hz, H-10), 6.63 (1H, brs, H-6), 2.50 (2H, brs, H_2-2′), 1.23 (18H, brs, 9 x CH_2), 0.84 (3H, t, *J*=6.2 Hz, Me-12′); ^{13}C NMR (DMSO-d_6): δ 181.07 (C-15), 170.27 (C-1′), 160.15 (C-1), 157.84 (C-2), 155.06 (C-8), 150.69 (C-14), 147.40 (C-12), 149.73 (C-13), 137.62 (C-5, C-7), 133.23 (C-3),130.36 (C-6,C-13), 129.93 (C-9,C-10); 115.57 (C-4), 51.21 (C-2), 33.46 (C-3), 29.85 (7xCH_2), 22.45 (CH_2), 14.15 (Me-12′).+ve ESI MS *m/z*: 452 $[M]^+$ ($C_{27}H_{32}O_6$).

Antifungal activity assay

The antifungal activity was performed on *Aspergillus flavus* (MTCC-277), *Candida albicans* (MTCC-3958) and *Penicillium citrinum* (MTCC-3395). For bioassay, a fungal suspension in sterile normal saline was prepared. An aliquot of 1.5 ml was uniformly seeded on the malt extract media (15 ml, 4 cm thickness) in Petri dishes, left aside for 15 min and excess was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured into culture media using sterile cork borer (6 mm). Concentrations of 1, 5, 10 and 20 mg/ml of the methanol extract and 25, 50, 100 and 200 µg/ml test compounds were prepared in dimethyl sulphoxide (DMSO). The standard drug Fluconazole was obtained from Cipla Labratories. The plates were then incubated at 30°C for 48 hrs. After incubation, bioactivity was determined by measuring the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls included solvent without plant extracts/tested compounds, although no antifungal activity was noted in the solvent employed for the test.

RESULTS AND DISCUSSION

Compound **7,** named coriarianaphthyl ether, was obtained as light brown amorphous powder from $CHCl_3$-MeOH (49:1) eluents. It showed UV absorption maxima at 217 and 274 nm characteristic of conjugated system. Its IR spectrum showed absorption bands for hydroxyl group (3450 cm^{-1}) and aromatic nucleus (1541, 1219, 890 cm^{-1}). The ESI mass spectrum of **7** showed a molecular ion peak at *m/z* 176 corresponding to molecular formula of a naphthalene derivative $C_{11}H_{12}O_2$. The ^1H NMR of **7** exhibited two one-proton double doublets at δ 8.03 (*J*=8.7, 3.0 Hz) and 7.03 (*J*=9.1, 2.9 Hz) assigned to *ortho-ortho* and *ortho-meta* coupled H-9 and H-6 aromatic protons, respectively. Two *ortho*-coupled

protons appearing as doublets at δ 7.96 (*J*=9.5 Hz) and δ 7.30 (*J*=9.5 Hz) were ascribed to H-2 and H-3 protons, respectively. The H-7 and H-8 aromatic protons appeared as one-proton multiplets at δ 6.79 and 6.50, respectively. A two-proton broad signal at δ 3.70 and another three-proton broad signal at δ 3.41 were due to oxygenated methylene and methoxy protons, respectively. The ^{13}C NMR of **7** exhibited signals for C-11 hydroxymethylene carbon at δ 67.20 and methoxy carbon at δ 51.91. The oxygenated aromatic carbon C-1 appeared at δ 166.68 whereas remaining aromatic carbons of the naphthalene ring resonated between δ 145.78-108.78. The HMBC spectrum of **7** showed interactions of C-1 with H-2 and OMe; C-4 with H-3 and H_2COH; and C-10 with H-8 and H-9. On the basis of the foregoing discussion the structure of **7** has been elucidated as 1-methoxy-4-hydroxymethylenenaphthalene.

Compound **8,** named coriariaoic acid, was obtained as brown crystals from $CHCl_3$-MeOH (97:3) eluents. It gave effervescences with sodium bicarbonate solution suggesting acidic nature of the compound and responded positively to the DNP test for carbonyl group. Its IR spectrum showed characteristic absorption bands for carboxylic group (3144, 1690 cm^{-1}), carbonyl group (1710 cm^{-1}) and aromatic nucleus (1541 cm^{-1}). It exhibited UV maxima at 294 nm characteristic of conjugated moiety. The ESI mass spectrum of **8** showed a molecular ion peak at *m/z* 194 corresponding to molecular formula $C_{10}H_{10}O_4$. Its ^{1}H NMR spectrum exhibited a downfield D_2O exchangeable signal integrating for one-proton at δ 12.27 due to carboxylic proton. A one-proton broad signal at δ 9.20 was assigned to aldehydic proton. The aromatic proton H-3 and H-6 resonated as *para*-coupled broad signals at δ 8.84 and 6.91 whereas two three-proton broad signals at δ 3.36 and 2.50 were assigned to methoxy protons and Me-9 protons, respectively. The ^{13}C NMR spectrum of **8** showed signals for aldehydic carbon at δ 203.51 (C-8), carboxylic carbon at δ 179.83 (C-7), aromatic carbons from δ 161.20 to 122.81, methoxy carbon at δ 55.87 and methyl carbon at δ 17.97 (C-9). The HMBC spectrum of **8** showed correlations of C-7 with H-6; C-2 with H-3 and OMe; C-4 with H-3 and H-8; and C-5 with H-6 and H_3-9. On the basis of chemical reactions and spectral data analysis the structure of **8** has been elucidated as 2-methoxy-5-methyl benz-4-al-1-oic acid. It is a new aromatic compound.

Compound **9,** named coriarianthracenyl ester, was obtained as brown crystals from chloroform-methanol (19:5) eluents. Its IR spectrum exhibited absorption bands for hydroxyl groups (3448, 3380 cm^{-1}), ester group (1725 cm^{-1}), carboxylic group (3250, 1695 cm^{-1}) and aromatic moiety (1541, 1075, 990 cm^{-1}). Aromaticity in the compound was supported by the UV absorption maxima at 266, 294 and 358 nm characteristic of a conjugated system. The ESI mass spectrum of **7** displayed a molecular ion peak at *m/z* 452, which corresponded to molecular formula of an anthracene ester, $C_{27}H_{32}O_6$. The ^{1}H NMR of **9** exhibited a downfield one-proton broad signal at δ 13.24 assignable to carboxylic proton. Two one-proton *ortho*-coupled doublets at δ 7.99 (*J*=8.1 Hz) and 6.96 (*J*=8.1 Hz) were accounted to H-3 and H-4 aromatic protons, respectively. A set of one-proton doublet at δ 7.05 (*J*=8.8 Hz) and 6.83 (*J*=8.8 Hz) was ascribed to *ortho*-coupled H-9 and H-10 protons, respectively. Four broad signals at δ 7.79 (1H), 6.63 (1H), 2.50 (2H) and 1.23 (18H) arouse due to H-13 and H-6 aromatic protons, methylene H_2-2′ protons adjacent to the ester group and other CH_2 aliphatic protons, respectively. The C-12′ primary methyl protons appeared as a triplet at δ 0.84 (*J*=6.2 Hz). The ^{13}C NMR spectrum of **9** exhibited signals for carboxylic carbon at δ 170.27 (C-1′), aromatic carbons from δ 160.15 to 115.57, methylene carbons between δ 51.21-22.45 and methyl carbons at δ 14.15 (C-12′). Alkaline hydrolysis of **9** yielded lauric acid. The HMBC spectrum of **9** exhibited correlations of C-2 with H-3 and H-4; C-8 with H-9; C-15 with H-10; and C-1′ with H_2-2′. On the basis of the foregoing discussions the structure of **9** has been elucidated as 1-dodecanoxy-2, 8-dihydroxy-anthracene-15-oic acid. This is a new anthracenyl ester isolated from a plant source.

Compounds **1-6** are the known aromatic compounds identified as *n*-tetracosane **(1)**, *n*-pentacosane **(2)**, anise alcohol **(3)**, *p*-hydroxybenzyl alcohol **(4)**, methyl lawsone **(5)** and 2-hydroxymethylenenaphthaquinone **(6)** respectively, on the basis of spectral data analysis and chemical reactions.

The antifungal activity results indicated that methanolic extract and all the three new compounds could reduce the growth of the fungus strains and the methanolic extracts of the plants were found to be effective against the selected strains of the fungi at all used concentrations. The compound **8** was effective

against both *A. flavus* and *C. albicans* at the lowest tested concentration of 25 µg/ml. It showed comparable results with that of standard at higher tested concentrations against *A. flavus* but in case of *C. albicans* the activity was lower than that of the standard drug. The compound **7** was found to be ineffective against *A. flavus* at lowest tested concentration of 25 µg/ml but showed comparable activity at higher concentrations with that of standard drug. However, it was found to be ineffective against *C. albicans* and *P. citrinium* at all the tested concentrations. The compound **9** was found to be active against all the tested fungal strains at all the concentrations (Table 1).

CONCLUSION

The findings of the present work have revealed that the seeds of *R. coriaria* are rich in phenolic compounds and the isolated aromatic compounds showed antifungal activity against *C. albicans* and *A. flavus*.

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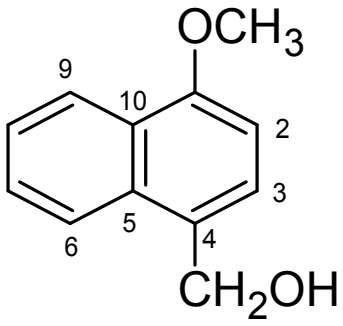
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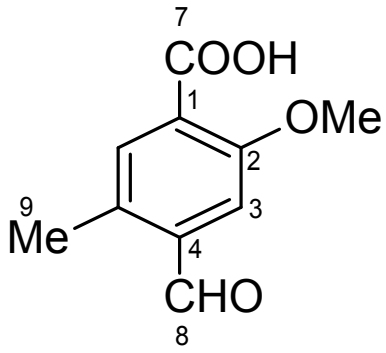
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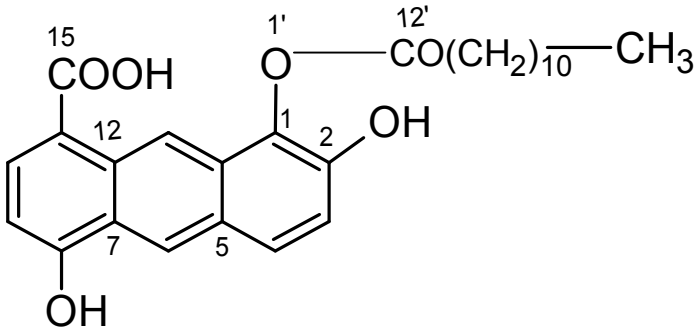
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Table 1: Antifungal activity of methanol extract and isolated compounds of *Rhus coriaria*

S.No.	Sample	Concentration	Mean zone of inhibition (mm)		
			Aspergillus flavus	*Candida albicans*	*Penicilium citrinium*
1.	Methanolic extract (mg/ml)	1	14	13	10
		5	15	16	10
		10	15	17	11
		20	18	19	13
2.	Coririanaphthyl ether (**7**) (µg/ml)	25	-	-	-
		50	12	-	-
		100	13	-	-
		200	13	-	-
3.	Coriarioic acid (**8**) (µg/ml)	25	10	12	-
		50	10	12	-
		100	11	12	-
		200	11	13	-
4.	Coriariacthracenyl ester (**9**) (µg/ml)	25	12	11	10
		50	12	11	10
		100	12	18	11
		200	13	19	12
5.	Fluconazole	30	19	18	19

Fluconazole was used as a standard compound (mg/ml for methanolic extract and µg/ml for **7**, **8**, **9**)

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