



Evaluation of Antimicrobial Activity of Some Compounds Isolated from *Rhamnus cathartica* L

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ABSTRACT

A new anthraquinone derivative, 1,8-dihydroxy-2-[(z)-4-methylpenta-1,3-dien-1-yl] anthraquinone (1), together with known anthraquinone compounds were defined to be, 2-acetyl-3,8-dihydroxy-6-methoxy-anthraquinone (2), emodin (3), glucofrangulin A (4) and phenanthrene derivative named dendrochrysanene (5), lactone compounds named [β -sorigenin (6) and geshoidin (7)], xanthone compound termed pruniflorone H (8), oxanthrone compound named rumejaposide I (9), flavonol compounds called [kaempferol (10) and quercetin (11)] have been isolated from the methanolic extract of *Rhamnus cathartica* L. All isolated compounds were obtained for the first time from the genus *Rhamnus cathartica* L. except emodin; they were subjected to antimicrobial activity test; compounds (1), (3), (9) exhibited antibacterial activity [G+ve, G-ve] and anti-yeast activity, whereas, compounds (5), (11) exhibited antibacterial activity [G+ve and G-ve]. But, compounds (2), (4), (7), (10) exhibited antibacterial activity G-ve while compounds (6), (8) exhibited antibacterial activity G-ve and anti-yeast activity. The isolated compounds were confirmed by detailed analysis of their ¹H, ¹³CNMR, mass spectral data and by the results of hydrolytic cleavage.

Keywords: Plant extract, *Rhamnus cathartica* L., antimicrobial activity, flavonol.

INTRODUCTION

Rhamnaceae, is a large family of flowering plants, mostly trees, shrubs and some vines. The family contains 50-60 genera and approximately 870-900 species. Rhamnaceae have a worldwide distribution, but are more common in the subtropical and tropical regions. Economic uses

of Rhamnaceae are chiefly as ornamental plants¹. The wood of *Rhamnus* was also the most favored species to make charcoal for use in gun powder before the development of modern propellants. Some *Rhamnus* species showed insecticidal activity², anticancer³, antimicrobial⁴, antioxidant and free radical-scavenging activity^{5,6}. Anthraquinones⁷⁻¹¹, anthrones⁷, flavonoides^{5-7,12},

anthracene derivatives¹³, geshoidin, sorigenin glycoside, chrysophanol, physcion, musizin¹⁴, emodin^{10,15}, polyphenolic¹⁶, procyanidin glycoside¹⁷ and essential oil¹⁸ are previously isolated from different *Rhamnus* species. *Rhamnus cathartica* (buckthorn), is native to Europe, northwest Africa, and western Asia. It is a deciduous shrub or small tree, the seeds and leaves are considered toxic to humans and animals, causing stomach cramps and laxative effects. The chemical compounds responsible for this laxative effect are anthraquinone and emodin, it was effective against various cancer disease tonic, cathartic, diuretic¹⁹. Display quotations of over 40 words, or as needed. Microbial diseases such as tuberculosis, candidiasis, cryptococcosis and salmonellosis are examples of known infectious diseases that have been on the increase in the recent history due to HIV/AIDS deadly disease. Resistance to antibiotics such as norfloxacin, ciprofloxacin and amoxicillin clavulanic acid by *Pseudomonas aeruginosa* and enterohemorrhagic *Escherichia coli* has been recognized²⁰. Multidrug resistance poses serious challenges to the medical field and infections caused by multi-resistant bacteria especially in the intensive care units pose a huge problem²¹. Use of plant products for the control of human diseases has certain advantages besides being cheap to produce; they are biodegradable and readily available. Effective plant extracts can combat human pathogenic bacteria without toxic side effects and environmental hazards²². Our interest in this paper was evaluation the antimicrobial activity of *R. cathartica* L. extract and the isolated compounds, it also discuss the structural elucidation of the isolated compounds by using different spectroscopic methods.

EXPERIMENTAL

Plant material

The plant was collected from Giza zoo garden, Egypt at September. The plant was confirmed by Dr. Mohamed El-Gibaly, Lecturer of Taxonomy and Consultant for Central Administration of Plantation and Environment. Voucher specimens were preserved in the Herbarium, Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt.

MATERIAL AND METHODS

¹HNMR analyses were run on JEOL GLM 500 MHz spectrometers relative to TMS in DMSO-d₆ and Me₂CO-d₆. ESI-MS was measured on a Finnigan TSQ 700 GC/MS equipped with a Finnigan electrospray source. Paper chromatography sheets Whatman No.1 (Maidstone, England), the chromatograms were visualized under UV light Vilber Lourmat (VL-6LC France) at 254 and 365 nm. Melting point was detected on (SMDP3 Stuart Scientific UK). Column chromatography was performed using polyamide 6S (Riedel de Darmstadt, Germany) as a stationary phase within a glass column 120 x 8 cm and on Sephadex LH-20 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden). TLC was applied on TLC plates with silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany). The solvent systems used were CHCl₃:MeOH (9:1) (system I), n-BuOH-AcOH-H₂O (4:1:5, upper layer) (system II), 15 % aqueous AcOH (system III) and EtOAc:MeOH:H₂O (4:3:0.8) (system IV). The authentic flavonoids and sugars were obtained from Department of Medicinal Chemistry, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt.

Equipment and chemicals for antimicrobial assays

Low temperature incubator SHEL-LAB model 2005 sheld on manufacturing. Inc, NUAJARE Biological safety cobient, LABSCO oven Laboratory supply company, Olmon and cokg Germany, Autoclave la Astell Hearson Germany, Refregerator Toshiba (no frost model FR-GF40P). Nutrient agar medium (LAB M, UK), sucrose (Oxford), NaNO₃ (s.d.fine chem. Ltd), MgSO₄ (s.d.fine chem. Ltd), KCl (s.d.fine chem. Ltd), FeSO₄ (s.d.fine chem. Ltd), K₂HPO₄ (MERCK), agar-agar bacto (s.d.fine chem. Ltd), *Staphylococcus aureus* (ATCC 6538-P), *Cadida albicans* (ATCC 27853), *Escherichia coli* (ATCC 10231), and *Aspergillus niger* (NRRL A-326). All the test microbes were obtained from the culture collection at Microbial Chemistry Department, National Research Center.

Extraction and isolation

The plant material was dried in shade, powdered and subjected to extraction and stored in air tight container for further use. The air-dried,

powdered *R. cathartica* L. (2 kg) were extracted five times with 85% methanol (10 L), under reflux (70 °C) for 7 days, and the combined methanol extract (300 gm) was defatted with n-hexane (2 L), chloroform (3 L), ethyl acetate (2.5 L) then the residual extract was dissolved in deionized water (200 ml), then the salt was removed by adding excess methanol (3 L), and filtered. The filtrate was dried to give (90 gm) extract, that was subjected to polyamide column chromatography (120 X 7 cm, 350 gm) using a consecutive elution with H₂O, H₂O:MeOH ratio, to pure MeOH elution system which afford 120 fraction. On the bases of comparative paper chromatography (Co-PC) with the use of 1% FeCl₃, UV light or Naturstoff spray reagent for detection, the obtained 120 fraction were collected into 6 collective fractions (1 – 6). Fraction 1 (H₂O, 17 g) was found to be has no phenolic character with a deep brown color. Fractions 2, 3, 4, 5 and 6 were subjected to further silica gel and Sephadex LH-20 column chromatography, Fr. 2 (3.1 g) was subjected to a H₂O:MeOH (90:10) eluent system, and purified by precipitation by ethyl acetate; the latter subjected to Sephadex LH-20 column chromatography with H₂O:MeOH (85:15) to afford compound 4 (30 mg) and compound 8 (15 mg). Fr. 3 (3.9 g) was subjected to a column chromatography eluting with H₂O:MeOH with increasing ratio from (80 to 20), were then followed by repeated Sephadex LH-20 CC (H₂O:MeOH, 90:10) to give compound 3 (23 mg), compound 6 (19 mg) and compound 9 (33 mg). Fr. 4 (1.7 g) eluted with H₂O:MeOH (70.5:29.5) and then purified on Sephadex LH-20 CC then acetone to produce compound 1 (29 mg). Fr. 5 (3.9 g) was further isolated by chromatographed on silica gel column eluted with CHCl₃:MeOH (9.5: 0.5), then passage over a Sephadex LH-20 column by H₂O:MeOH (70:30) elution system and crystallized by ethanol to give compound 2 (31 mg), 5 (28 mg) and 7 (21 mg). Fr. 6 (2 g) was applied to purification with eluent H₂O:MeOH (60:40), then subjected to precipitation by acetone followed by crystallization by ethanol to afford compound 10 (17 mg) and 11 (19 mg). Sub fraction 6 contains flavonoids behavior (based on its chromatographic properties of colour change into orange with Naturstoff spray reagent and green with FeCl₃).

Acid hydrolysis

Compounds 4, 7 and 9 (about 8.0 mg

each) were separately refluxed with 10 mL of 2 M HCl (MeOH:H₂O, 1:1) at 100 °C for 4 h. After the MeOH was removed, the residue was diluted with H₂O and extracted with CHCl₃ (10 mL x 3). The CHCl₃ extracts were evaporated under reduced pressure and purified to obtain the aglycone which was identified by comparison with authentic samples, using TLC analysis with solvent system (Benzene:MeOH, 9:1). The aqueous layer was concentrated under vacuum to give a residue of the sugar fraction, then neutralized by NaHCO₃. The residue was dissolved in pyridine (0.1 mL) to obtain the sugar moiety which was compared with authentic sugar by using TLC plate with solvent system (EtOAc:MeOH:AcOH:H₂O, 12:4:2:3). The comparison with authentic samples showed that the hydrolysates were β-D-glucose, α-L-rhamnose for compound 4 and β-D-glucose for compounds 7 and 9.

Antimicrobial activity test

Disc agar plate method was done to evaluate the antimicrobial activity of methanol extract from *Rhamnus cathartica* L. plant and other different isolated pure compounds. The antimicrobial activities of 0.5-cm-diameter filter paper disc saturated with about 1mg of extract and 100 µg of compound sample were tested against four different microbial strains, i.e., *Staphylococcus aureus* (G+ve bacteria), *Escherichia coli* (G-ve bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (fungi). Both bacterial and yeast test microbes were grown on nutrient agar (DSNZ 1) medium (g/l): beef extract (3), peptone (10), and agar (20). Whereas fungal test microbe was grown on Szapek-Dox (DSMZ130) medium (g/l): sucrose (30), NaNO₃ (3), MgSO₄.7H₂O (0.5), KCl (0.5), FeSO₄.7H₂O (0.001), K₂HPO₄ (1) and agar (20). The culture of each microorganism was diluted by sterile distilled water to 10⁷ to 10⁸ CFU/ml to be used as inoculum. 1ml of the previous inoculum was used to inoculate 1l of agar medium (just before solidification) then poured in Petri-dishes (10cm diameter containing 25ml). Discs (5 mm diameter) were placed on the surface of the agar plates previously inoculated with the test microbe and incubated for 24 h for bacteria and yeast but for 48 h for fungus at 37 and 30°C, respectively.

RESULTS AND DISCUSSION

The dry leaves of *Rhamnus cathartica* L. were exhaustively extracted with 85% methanol and defatted, then a chromatographic separation of the defatted methanol extract occurred over polyamide followed by Sephadex LH-20 and repeated silica gel column chromatography resulted in the isolation of a new anthraquinone derivative, 1,8-dihydroxy-2-[(z)-4-methylpenta-1,3-dien-1-yl]anthraquinone (1) (figure 1), together with known anthraquinone compounds were named to be, 2-acetyl-3,8-dihydroxy-6-methoxy- anthraquinone (2)²³, emodin (3)²⁴, glucofrangulin A (4)¹¹, one phenanthrene derivative called dendrochrysanene (5)²⁵, two lactone compounds namely [β -sorigenin (6) and geshoidin (7)]²⁴, one xanthone compound termed pruniflorone H (8)²⁶, one oxanthrone compound named rumejaposide I (9)²⁷ and two flavonol compounds identified as [kaempferol (10) and quercetin (11)]²⁸. The structure of these compounds were elucidated by ¹H-NMR, ¹³CNMR, ESI-MS, IR spectroscopy and by comparison with spectroscopic data reported in literature. Table (2) showed that all isolated compounds [1-11] including the main methanol crude extract have –ve effect against *A. niger* fungi. All isolated compounds and methanol extract showed significant effect on G+ve bacteria. Bath methanol extract, compounds 1, 3, and 9

showed significant antibacterial effect against *Escherichia coli* (G-ve), *Staphylococcus aureus* (G+ve) and antiyeast *Candida albicans*.

Table 1: ¹³CNMR data of compound 1

No.	Compound 1
1	160.9
2	134.2
3	137.2
4	120.1
4a	132.2
5	120.3
6	135.6
7	125.1
8	162.4
8a	118.7
9	190.8
9a	113.9
10	185.0
10a	130.6
1'	119.8
2'	127.7
3'	122.2
4'	139.9
5'	26.2
6'	18.7

Table 2: Antimicrobial activities of the methanol extract from *Rhamnus cathartica* L. and its purified compounds

S. No.	Sample	Clear zone (Ømm)			
		<i>E. coli</i> *	<i>Staphylococcus aureus</i> **	<i>Candida albicans</i> ***	<i>A. niger</i>
Control	Methanol @	0	0	0	0
Crude ext.	Methanol ext.	15	13	16	0
1	Compd. 1	15	14	15	0
2	Compd. 2	0	13	0	0
3	Compd. 3	12	12	11	0
4	Compd. 4	0	14	0	0
5	Compd. 5	12	14	0	0
6	Compd. 6	0	15	12	0
7	Compd. 7	0	12	0	0
8	Compd. 8	0	15	12	0
9	Compd. 9	13	14	14	0
10	Compd. 10	0	14	0	0
11	Compd. 11	15	12	0	0

* (G-ve); ** (G+ve); ***yeast; @ Control

Compound 1 was obtained after further purification of fraction 4 (H₂O:MeOH, 70.5:29.5), it is isolated as a deep yellow powder, it gives Rf value 0.57 on paper chromatography in [15 % aqueous AcOH], and its M.P. 268 °C. The preliminary qualitative tests performed by Maobe *et al.*, 2013; indicated that the plant contains anthraquinone²⁹. Physical, chemical and spectroscopic data suggested the anthraquinone of compound 1. IR spectrum of compound 1 exhibited two strong vibration bands at γ_{\max} 3444, 3435 cm⁻¹ due to presence of two free hydroxyl groups at C-1 & C-8, two carbonyl bands at γ_{\max} 1725, 1680 cm⁻¹ and at γ_{\max} 1560 cm⁻¹ for aromatic ring³⁰. ¹H NMR spectroscopic data showed two singlet one proton each at δ H 12.53, 12.50 due to *peri* to carbonyl moiety suggested the presence of 9, 10-dioxygenated anthraquinone^{31,32,33}. It also displayed resonance of five aromatic protons at δ H 7.53 (1H, *d* (*J*=7.5 Hz), H-3), δ H 7.94 (1H, *d* (*J*=7.5 Hz), H-4), δ H 7.97 (1H, *d* (*J*=7.5 Hz), H-5), and δ H 7.99 (2H, *m*, H-6 & 7), δ H 12.50 (OH-8) and δ H 12.53 (OH-1)³⁴. ¹H NMR spectroscopic data displayed signals suggested presence of methyl penta-1,3-dienyl side chain, three olefinic protons at δ H 6.33 (1H, *d* (*J*=11.7Hz), H-1'), δ H 6.10 (1H, *d* (*J*=11.7Hz), H-3'), 6.82 (1H, *dd*, (*J*=11.7 & 11.6Hz), H-2') and two methyl singlet at δ H 1.61, 1.54³⁵. ¹³C NMR spectroscopic data [table 1] showed signals for twenty carbon atoms including fourteen aromatic carbon characteristic to anthraquinone compounds³⁶ and six carbons for side chain. ¹³C NMR spectroscopic data confirmed the presence of two carbonyl groups at δ c 190.8 (C-9), 185.0 (C-10), two carbons substituted with hydroxyl groups at δ c 160.9 (C-1) and 162.4 (C-8). It showed resonance of four olefinic carbons at δ c 119.8 (C-1'), 127.7 (C-2'), 122.2 (C-3'), 139.9 (C-4') and two methyl singlet at δ c 26.2 and 18.7. ESI-MS spectrum fragmentation showed a molecular ion peak at *m/e* 321 [M+H]⁺ which suggest

in addition to previous data that the molecular formula C₂₀H₁₆O₄. This data suggest that compound 1 is 1,8-dihydroxy - 2 - [(z) - 4 - methylpenta -1,3- dien-1-yl] anthraquinone.

Structure-activity relationship (SAR)

As the metabolic responsible for antimicrobial activity; Compound 1 showed more antimicrobial activity than the other compounds. Interestingly this compound possesses an additional hydroxyl group at C-8, which might suggest that the structural fragment with a carbonyl and two β -hydroxyls at a linear position in anthraquinones may be an important pharmacophore for the antimicrobial bioactivities³⁷. The bioactive compounds affect the microbial pathogens (test microbes in five main ways depending upon the kind and class of compound, i.e. flavonoid, terpenes, alkaloid etc., these effects are inhibition of cell wall synthesis (most common mechanism), inhibition of protein synthesis (translation) (second largest class), alteration of cell membranes, inhibition of nucleic acid synthesis, antimetabolite activity. Anthraquinones or quinones are aromatic in nature with two ketone substitution. They are ubiquitous in nature and are highly reactive. It has been investigated that anthraquinones, extracted from different species of Aloe, exhibited antibacterial activity by inhibition of nucleic acid synthesis in the Gram positive bacterium³⁸. In addition, quinones are known to complex with nucleophilic amino acids in proteins often leading to inactivation of protein (including enzymes) and loss of function³⁹. Several investigations have been dealt with the mode of action of flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase. Mori and colleagues showed that DNA synthesis was strongly inhibited by flavonoids, whilst RNA synthesis was most affected in *S. aureus*⁴⁰. B-ring of the flavonoids may play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and that this may explain the inhibitory action on DNA and RNA synthesis⁴⁰. Ohemeng *et al.* screened 14 flavonoids of varying structure for inhibitory activity against *Escherichia coli* DNA gyrase⁴¹. It was found that *E. coli* DNA gyrase was inhibited to different extents by quercetin, enzyme inhibition was limited to those compounds with B-ring hydroxylation^{41,42}.

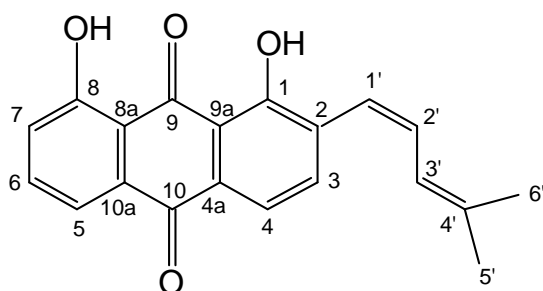


Fig. 1: Structure of compound 1

CONCLUSIONS

The methanolic extract of *Rhamnus cathartica* L. possess antimicrobial activity due to it has high contents of anthraquinone derivatives and flavonols. Chromatographic fractionation afforded eleven compounds belong to anthraquinone, phenanthrene, lactone, xanthone and flavonol. All isolated compounds were subjected to antimicrobial activity test; compounds (1), (3), (9)

exhibited antibacterial activity [G+ve, G-ve] and anti-yeast activity, whereas, compounds (5), (11) exhibited antibacterial activity [G+ve and G-ve]. But, compounds (2), (4), (7), (10) exhibited antibacterial activity G-ve while compounds (6), (8) exhibited antibacterial activity G-ve and anti-yeast activity. All compounds include methanol extract give negative effect against *A. niger* fungi. So we recommended that *Rhamnus cathartica* L. is suitable for using as antimicrobial plant.

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