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Two new antioxidant anthraquinones namely Obtusifolate A and B from *Rumex obtusifolius*

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Abstract

According to folk medicine *Rumex obtusifolius* 's root has a prominent detoxifying result on the liver and is used against fever, jaundice, and as an anti-anemic tonic. The roots are laxative and the leaves of this Rumex are used against hepatic, dermatological and eye problems. The diverse medicinal applications of *R. obtusifolius* and its genus have prompted us to investigate the constituents of the said plant. In bio-guided isolation Two new anthraquinones namely sec-butyl 1, 8-dihydroxy-5-methyl-3-(4'-(5"methylbut-4"-enoyl)-2'-(2"oxopropyl)-6-tert-pentyl-anthraquinone-2-carboxylate (Obtusifolate A) and methyl 1, 8-dihydroxy-5-methyl-3-(2'methyl-4'-(2"methylbut-1"-enoyl)-6-tert-pentyl-anthraquinone-2-carboxylate (Obtusifolate B) were isolated from *Rumex obtusifolius*. Their structures were identified by chemical and modern spectroscopic techniques i.e. ^{1}H and ^{13}C NMR, UV, IR and mass spectrometry. Both the compounds showed excellent antioxidant activities in the DPPH radical scavenging assay. Our findings provide evidence that *R. obtusifolius* is a potent source of natural antioxidants, and thus justified its uses in folkloric medicines.

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Introduction

Rumex obtusifolius (Polygonaceae) is a plant extensively studied because of its traditional uses in medicine in several countries of South America. According to folk medicine this plant's root has a prominent detoxifying result on the liver and is used against fever, jaundice, and as an anti-anemic tonic. The roots are also laxative. In addition, the leaves of this Rumex are used against hepatic, dermatological and eye problems. They are functional in the relief of furuncles, bruises and are also used as antiseptic and as scar healer (Girault, L. (198)Among the isolated compounds from this plant we can find a glycopyranoside: 6-O-malonyl-β methyl-glucopyranoside (Kasai *et al.,* 1981). Another effort has precise direction of the isolation and identification of amino acid plastocyanin (Haslett *et al.,* 1978). Isolation of anthroquinones has also been reported from many types of *Rumex* (Harborne & Mokhtari, 1977; Munavu *et al.,* 1984; Midiwo *et al.,* 1985; Hasan *et al.,* 1995*;* Demirezer *et al.,* 2001)), naphthalene glycosides (Demirezer *et al.,* 2001*)*, α-naphtols (Ibáñez-Calero *et al.,* 2009), simple naphtalenes, tannins, naphtoquinone derivatives (Demirezer & Kuruüzüm *et al.,* 1997, Suri *et al.,* 1978), anthracene derivatives (Fassil *et al.,* 1985) and flavonoid glycosides (Hasan *et al.,* 1995). Anthraquinones are one of such compounds which occur naturally in insects and some plants, lichens, fungi where they serve as a essential skeleton for their pigments. Anthroquinones are study of attention due to their extensive range of applications (Mueller *et al.,* 1999). Anthraquinones derivatives are confirmed to possess numerous biological activities such as anti-inflammatory (Gautam *et al.,* 2010), antifungal (Agarwal *et al.,* 2000), antioxidant (Iizuka *et al.,* 2004), anticancer (Zhou *et al.,* 2006), and genotoxicity and anti-genotoxicity (Mueller *et al.,* 1999). The diverse medicinal applications of genus *Rumex* have prompted us to investigate the constituents of *R. obtusifolius*.

Herein, we reported the isolation, separation and identification two new anthraquinones known as sec-butyl 1, 8-dihydroxy-5-methyl-3-(4'-(5"methylbut-4"-enoyl)-2'-(2"oxopropyl)-6-tert-pentyl-anthraquinone-2-carboxylate and methyl 1, 8-dihydroxy-5-methyl-3-(2'methyl-4'-(2"methylbut-1"-enoyl)-6-tert-pentyl-anthraquinone-2-carboxylate.

Materials and methods

All the solvents were distilled before use. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Brucker spectrometer operating at 500 MHz and 250MHz respectively, using $CDCl_3$ as a solvent, $CDCl_3$ gives at δH 7.26 ppm and at δC 77.00 ppm. Chemical shift values are reported relative to TMS. Silica gel (230–400 mesh) for column chromatography and aluminum sheets coated with silica gel 60 F 245 (20 cm × 20 cm) 0.2 mm thick; were used for TLC (E. Merck). Visualization of the spots on TLC plates was carried out by UV at 254 and 366 nm and by developing reagent (H_2SO_4 25%). JASCO DIP-360 polarimeter was used to determine optical rotation. Shimadzu 460 and Shimadzu UV-2401PC spectrometers were used for IR and UV spectra determination respectively.

Plant collection, identification and grinding

The whole plant excluded fruit and seed of *R. obtusifolius* (Polygonaceae) was collected from Bannu, Khyber Pakhtoon Khawa, Pakistan during start of December 2015and identified by Prof. Abdur Rehman, Botany Department, GPGC Bannu. The plant was washed, shade dried. It was then grinded into powders using electrical grinder.

Extraction, fractionation Isolation and purification

The powders of the grinded plant were extracted with EtOH for 14 days at room temperature. EtOH extract was evaporated by rotary to obtain thick gummy crude. The crude was successively fractionated with *n*-hexane, dichloromethane (DCM), ethylacetate. The DCM fraction was Sub-fractionated into F_1, F_2, F_3, F_4, F5 and F_6 by CC as shown in Table 2. For bioassay guided isolation study F4, F5 and F6 were proved to be the most active antibacterial, cytotoxic and antifungal agents (Khabir *et al.,* 2017).

So F_5 fraction was subjected to column chromatography over silica gel, eluting with n-hexane, n-hexane/DCM, DCM /AcOEt, and MeOH in increasing order of polarity. Eluting with the solvent system n-hexane/DCM 1:9 to afford A and B was obtained in semi-pure form from the column by using DCM/AcOEt 9:1 as eluent. It was purified by pencil chromatography.

Radical scavenging activity (RSA)

TLC-DPPH assay was used for qualitative analysis of radical scavenging screening as described in literature (Takao, T, *et al.*,(1994), and for quantitative estimation of radical scavenging activity (RSA) according to the standard assay (Mahato, S. B., & Kundu, A. P, *et al.*,1994). In brief 2.5cm^3 of 0.04% DPPH radical solution in methanol was added to each sample solution (100×10^{-6}L) ranging from 192.50 to 6×10^{-6}g/cm^3.

The mixtures were vortex-mixed and placed in dark room for half an hour. The absorbance was recorded at 517nm using spectrometer. MeOH was used as baseline control and ascorbic and gallic acid as positive controls. RSA was calculated as decrease in absorbance (samples V DPPH standard solution). %RSA was measured by the following formula. % RSA= [(A-B)/A)] ×100, where A is absorbance of control and B is absorbance of sample.

Results and discussion

Identification test for multiple bond in A and B

Compound A was obtained as yellow amorphous solid.

The Compound was identified through UV-VISIBLE, IR, NMR and MS studies as an anthraquinone derivate.

Table 1. 1H-NMR ^{13}C-NMR (Chemical shift (δ) values) data of compouns A and B.

Chemical shift (δ) values of compound A			Chemical shift (δ) values of compound B		
A			B		
H/C	δ (H)	δ(C)	H/C	δ (H)	δ(C)
1-OH	12.31	165.42	1-OH	12.33	165.00
2	----	117.00	2	----	116.01
3	----	138.90	3	----	138.60
4	7.02s	124.50	4	7.02s	124.55
5	----	144.00	5	----	144.00
6	----	150.13	6	----	150.14
7	7.12s	144.40	7	7.12s	144.40
8-OH	12.13	159.00	8-OH	12.14	159.00
9,	-----	190.50,	9	-----	190.50
10	-----	181.00	10	-----	181.00
4a		134.04	4a	------	134.04
8a		114.20	8a	-----	114.20
9a		113.10	9a	-----	113.10
10a		133.15	10a	-----	133.15
11	2.38s	20.01	11	2.38s	20.00
12	----	34.50	12	----	34.50
13	1.28s	29.60	13,14	1.28s	29.60
14	1.28s	29.60	15	1.20q	37.6
15	1.20q	37.6	16	0.85d	9.00
16	0.85d	9.00	17	----	166.10
17	----	168.12	18	4.23s	51.60
18	4.29---4.20(m)	74.00	1'	----	139.50
19	1.35d	22.02	2'	----	136.70
20	1.75m	29.80	3'	7.66 d m-cop	130.00
21	.92--.96 t	8.01	4'	----	136.60
1"	3.62s	48.4	5'	7.72—7.74	127.80
2"	----	206.01	6'	7.56d o-cop	127.50
3"	2.13s	31.30	7'	2.47s	22.50

4"	----	192.10	1"	----	192.10
5"	6.69s	120.00	2"	6.69s	120.00
6"	----	133.10	3"	----	133.10
7"	1.65s	18.5	4"	1.65s	18.5
8"	1.70s	25.5	5"	1.70s	25.5
1'	----	138.70			
2'	----	132.30			
3'	7.66 d	129.40			
4'	----	136.60			
5'	7.72—7.74 dd	127.80			
6'	7.56d	127.50			

Compound A

Compound A was obtained as yellow amorphous sold. The Compound was identified an anthraquinone derivate as listed below.

UV-VISIBLE and IR of 'A'

The absorptions at (3441,3425), (1701–1728), (1590) and (1619) in cm^{-1} of the IR spectrum showed the presence of chelated hydroxyl grougs, ester groups, conjugated carbonyl groups of anthraquinone nucleus and benzene ring respectively. Absorption at 1715 cm^{-1} is due to non conjugated carbonyl group.

The UV spectra of A showed maximum absorptions at 211, 239, 252, 275, and 366 nm, attributed a highly conjugated system (Harding, W. W, *et al.,* 1999).

Table 2. Sub-fractionation of DCM fraction by passing DCM, n-Hexane and ethyl acetate in various proportions as eluent in Column Chromatography.

Sub-fractions	Eluent	
	% DCM	%n-hexane
F_1	0	100
F_2	25	75
F_3	50	50
F_4	75	25
F_5	100	0
F_6	100% ethyl acetate was eluted at last	

Mass spectrometry of 'A'

The molecular formula was determined through HREI-MS as $C_{39}H_{42}O_8$ (m/z =638.2880; calcd. 638.2870), which shows 15 degrees of unsaturation. Eight of them are eventually accounted for anthraquinone ring, 3 for the tri-substituted benzene ring; one was due to double bonds, two for ketonic and last one for ester carbonyls group.

1H-NMR spectrum of 'A'

Compound A decolorized $KMnO_4$ solution, which showed non-aromatic multiple bond(double or triple bond).

The 1H-NMR spectrum of compound A confirm alkene moiety (double bond) with a proton at (6.69s) together with two different Me group at (1.65s, 1.70s) attached to the same double bonded carbon. Two singlet's at (12.13s, HO-1, and 12.31s, HO-8) revealed the presence of two *peri*-positioned chelated hydroxyl groups (Schripsema & Dagnino *et al.,* 1996).

Two *peri*-positioned chelated hydroxyl groups together with two conjugated carbonyl groups(C-9, δ 190.50; C-10; δ 181.00) confirmed the presence of anthraquinone nucleus.

A pair of singlet appearing at (7.02s, 1H) and (7.12s, 1H) indicated a tetra-substituted 1,8-dihydroxy anthraquinone.

Table 3. %RSA of compound A and B from *Rumex obtusifolius*.

S. No	Compounds/standards	% DPPH inhibition±SEM
1	A	97.55±1.51
2	B	96.00±0.83
3	Gallic acid	93.00±0.29
4	Ascorbic acid	92.33±0.55

A mutiplet at (4.20-4.29ppm, 1H) corresponding to H-18 was attributed to the oxymethylene protons. Signals at (7.66d, H-3') due to meta coupling having small J values; (7.72-7.74dd, H-5') corresponding to ortho and meta coupling; (7.56d, H-6') for ortho coupling) showing another benzene ring. Signals at (2.38s H-11 &2.13s H-3'') having same height indicated Me groups directly attached to aromatic ring and to carbonyl group respectively.

A singlet at (3.62s, H-1'') was due to methylene group present b/w carbonyl and aromatic ring. A highest singlet in up field region (6H, 1.28s, H-13 and H-14) confirmed two methyl groups in same chemically environment. The up field signals (0.85—1.70ppm) were due to protons of various chains of alkyl groups listed in table 1.

^{13}C NMR spectrum of 'A'

^{13}C NMR spectrum determined through a DEPT (Distortionless Enhancement by Polarization Transfer) experiment shows 38 signals for 39 carbon atoms, in which 20 are for olefinic , four for carbonyl, and the remaining are for SP^3 carbons of methane, methylene and methyl groups.

The stereochemistry at the chiral center C-18 was determined by the alkaline hydrolysis of A that yielded an alcohol that could be identified as (R)-1-methyl-1-propanol through the sign of its optical rotation.

The most downfield signal at (206.01ppm) is assigned to non-conjugated carbonyl carbon. Other downfield signals at (190.50, 181.00) and (168.12) are assigned to the conjugated carbonyls of anthraquinone ring and ester carbonyl respectively. The enone portion C=O resonated at (192.10, C-4'').

The carbon signal appearing at (74.00) is attributed to the oxymethine carbon. The most important signals, which appears at (165.42, 159.00) are assigned for C-1 and C-8 to which chelated hydroxyl groups are attached. Another up field signal at 29.60ppm is due to C-13 and C-14, which confirmed two methyl groups carbons in almost same chemically environment.

Stereochemistry of 'A'

The stereochemistry at the chiral center C-18 was determined by the alkaline hydrolysis of A that yielded an alcohol that could be identified as (R)-1-methyl-1-propanol through the sign of its optical rotation.

Compound B

Compound B was also obtained as pale yellow amorphous solid.

UV-VISIBLE and IR of 'B'

The absorptions at (3441, 3425), (1701–1728), (1590) and (1619) in cm^{-1} of the IR spectrum showed the presence of chelated hydroxyl groups, ester groups, conjugated carbonyl groups of anthraquinone nucleus and benzene ring respectively.

The UV spectra of A showed maximum absorptions at 211, 252, 275, and 366 nm, attributed a highly conjugated system (Harding, W. W, *et al.*, 1999).

Mass spectrometry of 'B'

Through HREI-MS the molecular formula of compound B was determined as, $C_{34}H_{34}O_7$ molecular ion peak (m/z =554.2305; calcd.554.2296), which shows 14 degrees of unsaturation. Eight of them are accounted for anthraquinone ring, 3 for the tri-substituted benzene ring; one was due to double bonds, one for ketonic and last one for ester carbonyls group.

The double bond in B was also confirmed by Baeyer, s test and spectroscopy dada as for compound A.

[1]HNMR spectrum of 'B'

The [1]HNMR and [13]CNMR is similar to compound A with little differences as shown in table1. Two singlets (12.14s, HO-1, and 12.33s, HO-8) revealed the presence of two *peri*-positioned chelated hydroxyl groups (Schripsema & Dagnino *et al.*, 1996). Two *peri*-positioned chelated hydroxyl groups together with two conjugated carbonyl groups(C-9, 190.50; C-10,181.00) also confirmed the presence of anthraquinone nucleus in compound B.

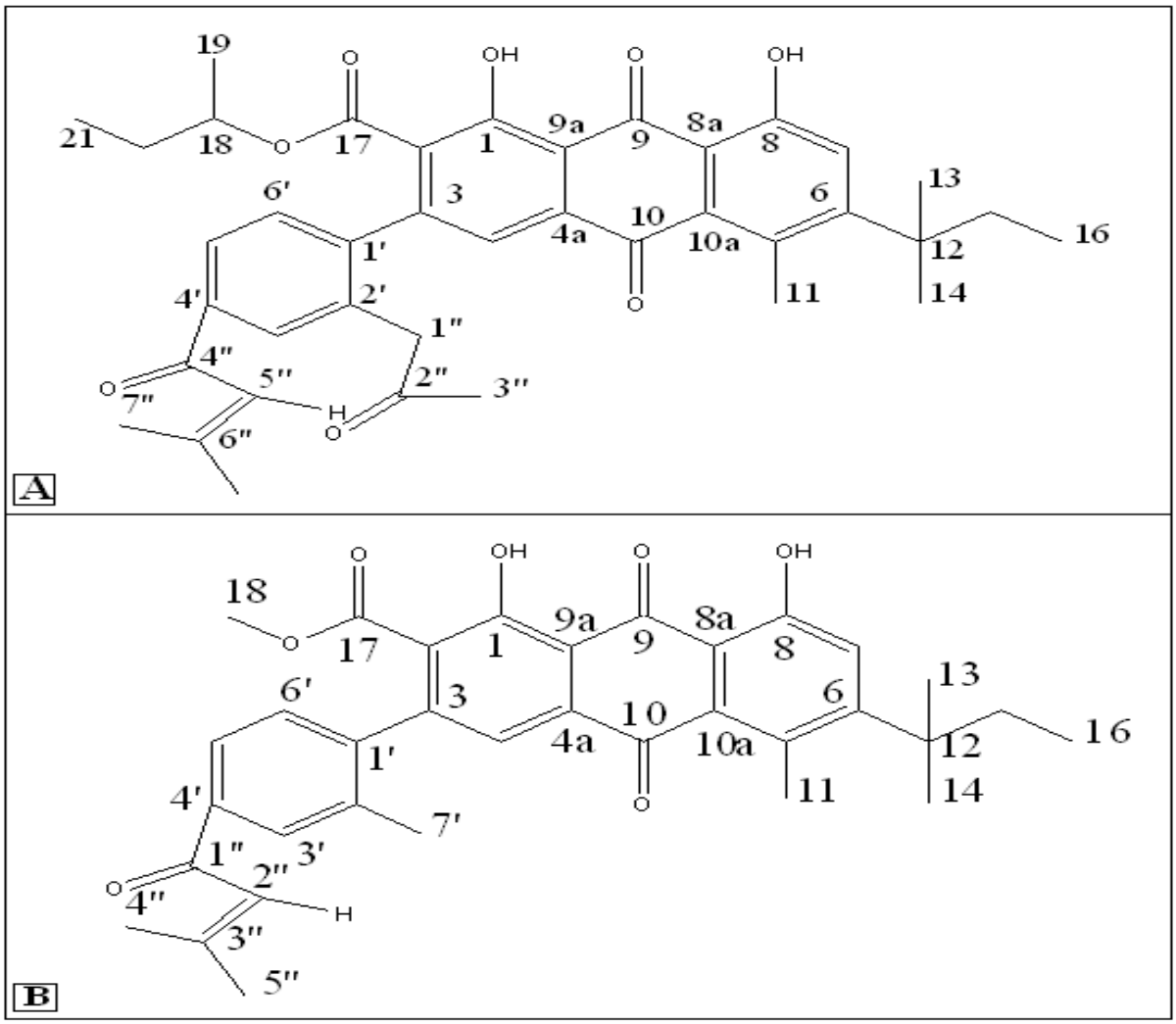

Fig. 1. Structure of compound A and B.

A pair of singlet appearing at (7.02s, 1H, H-4) and (7.12s, 1H, H-7) indicated a tetra-substituted 1,8-dihydroxy anthraquinone. A singlet at 4.23ppm (3H) corresponding to H-18 was attributed to the oxymethylene protons. Signals at {7.66d H-3' due to meta coupling having small J values; (7.72-7.74 dd H-5') corresponding to ortho and meta coupling; (7.56d H-6') for ortho coupling} showing another benzene ring as in compound B. Signals at (2.38s H-11 and 2.47s H-3") having same height indicated Me groups directly attached to aromatic rings. A highest singlet in up field region (6H, 1.28, H-13 and H-14) also confirmed two methyl groups in same chemically environment in compound B. The up field signals (0.85—1.70ppm) were due to protons of various chains of alkyl groups listed in table1.

[13]C NMR spectrum of 'B'

The [13]C NMR spectrum determined through a DEPT experiment shows 33 signals for 34 carbon atoms,

in which 16 are for olefinic , three for carbonyl, and the remaining are for SP^3 carbons of various methine, methylene and methyl groups. The most downfield signals at (190.50, 181.00) and (166.10) are assigned to the conjugated carbonyls of anthraquinone ring and ester carbonyl respectively. The enone portion C=O resonated at (192.10, C-1").

The carbon signal appearing at (51.60) is attributed to the oxymethyl carbon.

The most important signals, which appears at (165.00, 159.00) are assigned for C-1 and C-8 to which chelated hydroxyl groups are attached. Another up field signal at 29.60ppm is due to C-13 and C-14, which confirmed two methyl groups carbons in almost same chemically environment.

The different olefinic carbons resonated at (113.00—150.00) which are present in various chemical environments.

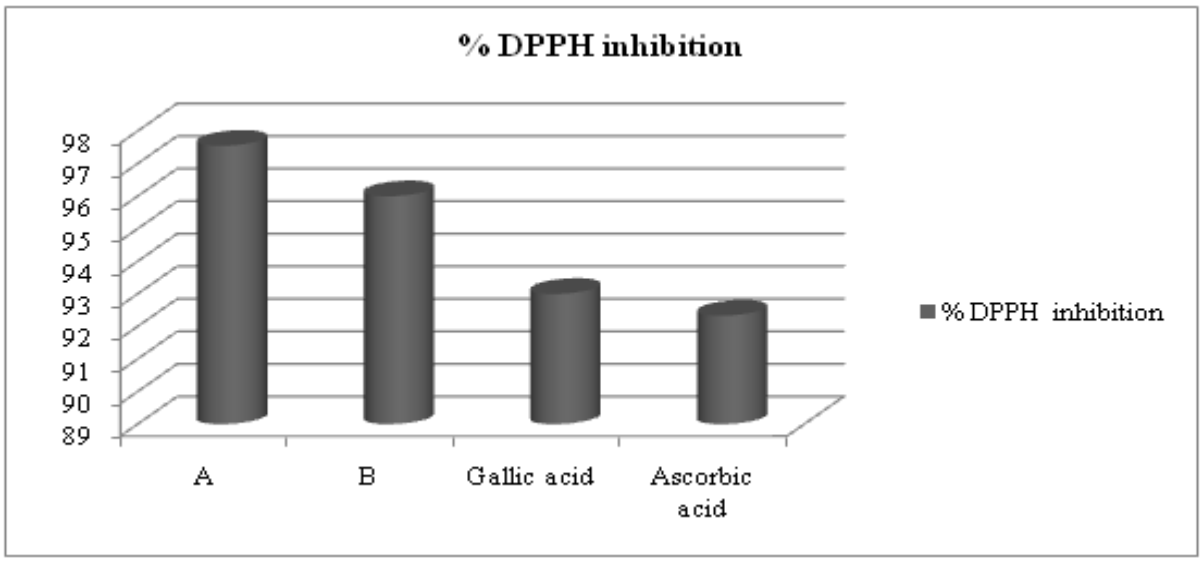

Fig. 2. % DPPH inhibibition zone of compound A and B.

%DPPH inhibition of compound A and B

The results of table 3 show prominent radical scavenging activity A & B. There is a very little difference in RSA b/w the two compounds. Both of the isolated compounds have greater radical scavenging property than ascorbic and gallic acid. %DPPH inhibition has been shown graphically.

Conclusion

The phytochemical studies of the medicinal plant *Rumex obtusifolius* were carried out by using modern chromatographic and spectroscopic techniques and methods. Two new compounds were obtained from the DCM soluble fraction, both of which have excellent antioxidant activities in the DPPH radical scavenging assay. *R. obtusifolius* is a common ingredient of traditional medicine in some parts of the world, therefore, further investigations on this plant are recommended to exploit its hidden medicinal importance.

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