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Two new antioxidant anthraquinones namely Obtusifolate C & D from *Rumex obtusifolius*

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Abstract

Rumex obtusifolius L. (Polygonaceae) is one of the most common wayside weeds and commonly known as broad-leaf dock. This plant is used for treatment of sores, tumors, blisters, burnsand cancer. It is also used as an antidote to nettle, depurative, astringent, tonic and laxative. Two new anthraquinones namely Ethyl 2-(18,18-dimethylbutanoyl)-1-methyl9,10-dioxo-5-(2-oxopropyl) anthraquinone-7-carboxylate (Obtusifolate C) and Ethyl 2-(16,16-dimethylbutanoyl) -4-methoxy, -1-methyl 9,10-dioxo, anthraquinone-7-carboxylate (Obtusifolate D) were isolated from *Rumex Obtusifolius*. The structures were elucidated by chemical and spectroscopic methods i.e. ^{1}H and ^{13}C NMR, UV, IR and mass spectrometry. Both the compounds showed significant antioxidant activities in the DPPH radical scavenging assay (RSA). The diverse medicinal importance of *Rumex obtusifolius* compels us to this work.

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Introduction

Rumex obtusifolius (*Rumex*) is a species widely studied b/c it is used in traditional medicine in a number of countries in S. America. According to traditional medicine root of this plant has a marked detoxifying effect on the liver and is used for treatment of fever, jaundice and as an anti-anemic tonic. This plant's root is laxative. The leaves of *Rumex obtusifolius* are used against eye, hepatic and dermatological infections. They are used for the relief of furuncles, bruises, and are also applied as scar healer and as disinfectant (Girault, L., 1984).

Among the compounds previously isolated from this species are 6-O-malonyl-β methyl-glucopyranoside (Kasai, T., Okuda, M. and Sakamura, S., 1981), amino acid plastocyanin (Haslett, Barry G., *et al.,* 1978). Isolation of demethylmacrosporine I, an anthraquinone derivative have been reported by (Ibáñez-Calero, S. L., Jullian, V. and Sauvain, M., 2009). Another effort has precise direction of the isolation and identification of amino acid plastocyanin (Haslett, B. G, T *et al.*,1978).

Rumex species contain anthraquinones (Khetwal, K. S., Manral, K., & Pathak, R. P. 1987, Liang, Heng-Xing, *et al.,* 2010), which showed various pharmacological properties, such as antioxidant (Demirezer, L. Ömür, *et al.,*2001), antitumor (Yang, Yang, *et al.,* 2013) and antimutagenicity (Lee, Nam-Jae, *et al.,* 2005) activities.

The World Health Organization of UN projected about eighty percent of the people in the developing countries depends on traditional medicine for primary health care requirements, of which a major ratio corresponds to plant extracted products (Salatino, A., Salatino, M. L. F., & Negri, G.(2007).

Here we present the bio-guided isolation of the active compounds from *Rumex obtusifolius*, its structure determination by various chemical and analytical techniques and their antioxidant activities. The aim of this work is to isolate new bioactive compounds from *Rumex obtusifolius* for free radical inhibition assay.

Materials and methods

Purification of the solvents was taken place by distillation. ^{13}C-NMR and^{1}H-NMR spectra were recorded with a Brucker spectrometer operating at 250 and 500MHz respectively, using $CDCl_3$ as a solvent. Chemical shift values were recorded relative to TMS. Silica gel (230–400 mesh) for column chromatography (CC) and aluminum sheets coated with silica gel 60 F 245 (20 × 20) cm 0.2 mm thick; were used for TLC. The spots on TLC plates was Visualized by UV at 254 and 366 nm and by ($H_2SO_4$25%) as developing reagent. Shimadzu UV-2401PC and Shimadzu 460 and spectrometers were used for UV and IR spectra recording respectively.

Collections, identification and grinding

The plants of *R. obtusifolius* was collected from Bannu, Khyber Pakhtoon Khawa (KPK), Pakistan during start of December and identified by Prof. Abdur Rehman, Botany Department, GPGC Bannu. The plant was washed with water shade dried and grinded into powders.

Extraction, fractionation and isolation

The powders were extracted with ethanol for 15 days at room temperature. The ethanol extract was evaporated 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 column chromatography as shown in Table No **3**.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_6 fraction was subjected to column chromatography over silica gel, eluting with n-hexane, n-hexane/DCM, DCM/AcOEt, AcOEt and MeOH in increasing order of polarity. Eluting with the solvent system AcOEt /DCM 1:1 to afford D and C was obtained in semi-pure form from the column by using DCM/AcOEt 1:2 as eluent. It was then purified by pencil chromatography.

Free radical scavenging activity

TLC-DPPH assay was used for qualitative analysis of radical scavenging screening as described in literature (Takao, *et al.*,1994), and for quantitative estimation of

RSA a standard assay was applied. (Mahato, & Kundu, 1994). Briefly 2.50cm^3 of 0.04% DPPH radical solution in MeOH was added to each sample solution (100×10^{-6}L) ranging from 192.50 to 6×10^{-6}gcm^{-3}. The mixtures were vortex-mixed and sited in dark room for 30min. The absorbance was taken at 517nm using spectrometer. Methanol was used for baseline control and ascorbic and gallic acid as positive controls. RSA was presented as decrease in absorbance (samples V DPPH standard solution). %RSA was measured by the following equation. %RSA=$[(A-A_1)/A)] \times100$, where A is absorbance of control and A_1 is absorbance of sample.

Result and discussion

Repeated column chromatography of the DCM-soluble fraction of the EtOH extract of *R. obtusifolius,* afforded compounds "C" and "D" as amorphous solids. Their structures were elucidated by UV, IR and NMR spectroscopy and mass spectrometry.

Table 1. NMR data (Chemical shift value= δ) of compounds C & D.

C			D		
H/C No	δ (H)	δ(C)	H/C No	δ (H)	δ(C)
8	7.74d	124.50	8	7.74d	124.50
7	-----	117.00	7	-----	117.00
6	7.72d	129.40	6	7.72d	129.40
5	----	132.30	5	----	150.13-----
4	7.55d	127.50	4	7.55d	127.50
3	7.57d	127.80	3	7.57d	127.80
2	------	136.60	2	------	136.60
1	------	144.00	1	------	144.00
9	------	181.00	9	------	181.00
10	-------	182.50	10	-------	182.50
1a	-------	134.04	1a	-------	134.04
8a	--------	133.15	8a	--------	133.15
4a	--------	132.00	4a	--------	132.00
5a	--------	132.50	5a	--------	132.50
11	--------	168.12	11	--------	168.12
12	4.23---4.27q	72.50	12	4.23---4.27q	72.50
13	1.28t	22.70	13	1.28t	22.70
14	3.91s	48.40	14	3.79s	56.60------
15	----	206.01	15	------	209.00
16	2.13s	31.30	16	-----	48.40
17	------	209.00	17	1.35s	22.02
18	-----	48.40	18	1.35s	22.02
19	1.35s	22.02	18	1.42—1.48q	34.50
20	1.35s	22.02	20	0.93---0.97t	9.00
21	1.42—1.48q	34.50	21	2.39s	20.01
22	0.93---0.97t	9.00			
23	2.39s	20.01			

Compound C (Obtusifolate C)

Compound C was isolated as an orange gummy solid and gave red solutions on reduction in alkaline solution (in aqueous sodium hydroxide, which distinguished anthraquinone from benzoquinones and naphthoquinones (Bizuayehu, Z, 2008).

UV and IR spectra of "C"

In UV spectra intense benzenoid absorption at 240-260 nm, medium absorption at 320-330 nm a strong quinonoid electron transfer band at 270-290 nm accompanied by a weak quinonoid absorption band at 405 nm showed anthraquinones skeleton.

The absorptions at (1701–1728) and (1590) in cm^{-1} of the IR spectrum showed the presence of ester group and conjugated carbonyl groups of anthraquinone nucleus. Absorption at 1715 cm^{-1} is due to non conjugated carbonyl group(C-15).

Mass spectrometry of "C"

The molecular formula was determined through HREI-MS as $C_{27}H_{28}O_6$ (m/z= 448.1886; calcd.448.1876), which shows 11 degrees of unsaturation. Eight of them are eventually accounted for anthraquinone ring, two for ketonic and last one for ester carbonyls group.

Table 2. Percentage of RSA of compound C and D from *Rumex obtusifolius*.

S.No	Compounds/standards	% DPPH inhibition ± SEM
1	C	80.12
2	D	79.00
3	Gallic acid	93.00
4	Ascorbic acid	92.33

^{1}HNMR spectrum of "C"

Two pair of doublets appearing at (7.55, 7.57) and (7.72, 7.74) ppm due ortho and meta coupling respectively indicated a tetra-substituted anthraquinone. Signals at (2.39s H-23 &2.13s H-16) having same height indicated methyl groups directly attached to aromatic ring and to carbonyl group respectively. A singlet at (3.91s, H-14) was due to methylene group present between carbonyl and anthraquinone ring. A highest singlet in up field region (6H, 1.35s, H-19 and H-20) confirmed two methyl groups in same chemically environment.

Table 3. Sub-fractionation of DCM fraction.

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.	

The various up field signals at (0.96-1.28) pmm were due to protons of various chains of alkyl groups listed in Table 1.

^{13}C NMR spectrum of "C"

^{13}C NMR spectrum shows 26 signals for 27 carbon atoms, in which 12 are for olefinic, four for carbonyl, one for ester and the remaining are for SP^3 carbons of methine, methylene and methyl groups.

The most downfield signals at 209.00ppm and 206.01ppm are assigned to C-17 and C-15 carbonyl carbons. Other downfield peaks at (181.00, 182.50) and (168.12) are assigned to the carbonyls carbons of anthraquinone ring and ester carbonyl carbon respectively.

The signal at 72.50ppm is attributed to the oxy-methylene carbon. Another up field signal at 22.02ppm assigned to C-19 and C-20 confirmed two methyl groups carbons in almost same chemically environment. All the above evidences confirmed the structure of compound 1.

Compound D (Obtusifolate D)

Compound Dwas isolated as a brown amorphous solid and also gave red solutions on reduction in alkaline solution (in aqueous sodium hydroxide, which distinguished anthraquinone from benzoquinones and naphthoquinones (Bizuayehu, Z. 2008).

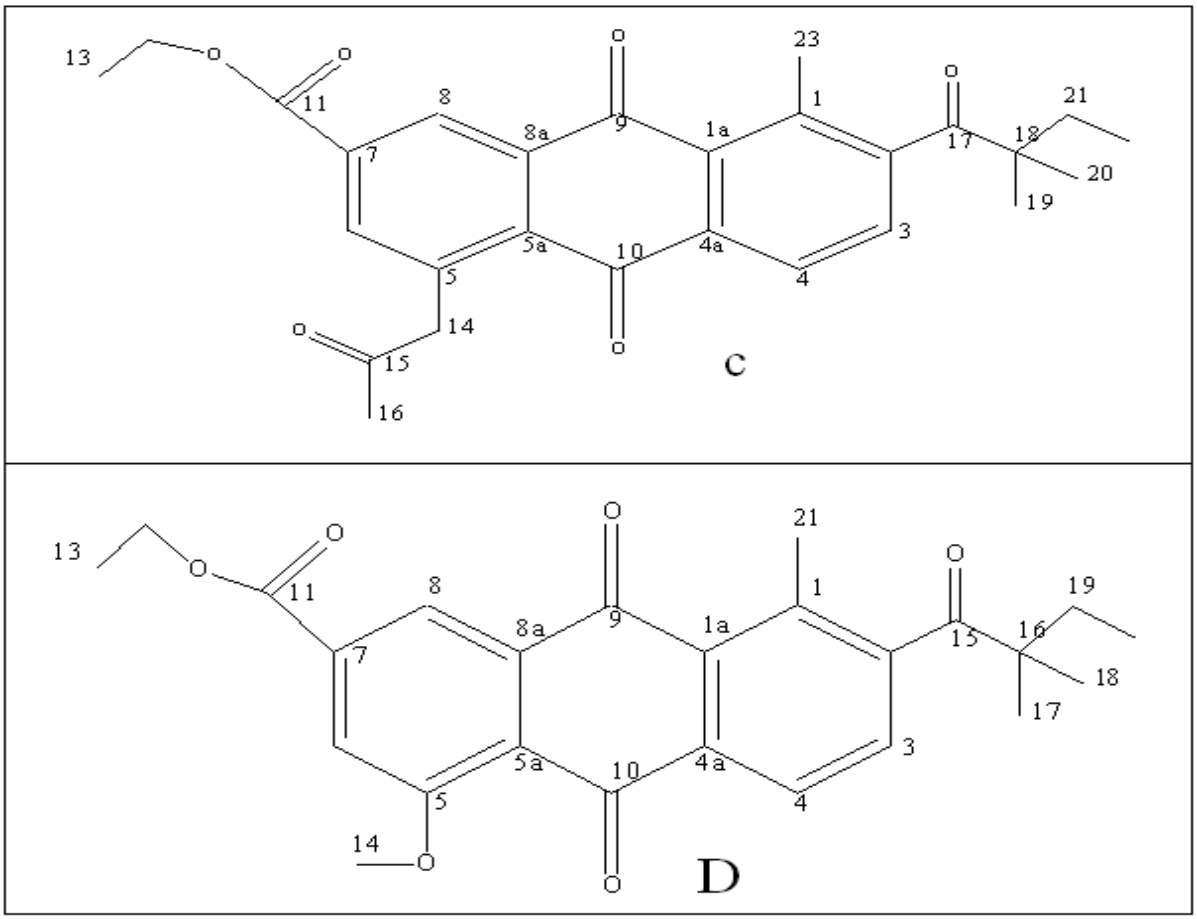

Fig. 1. Structures of compound C & D.

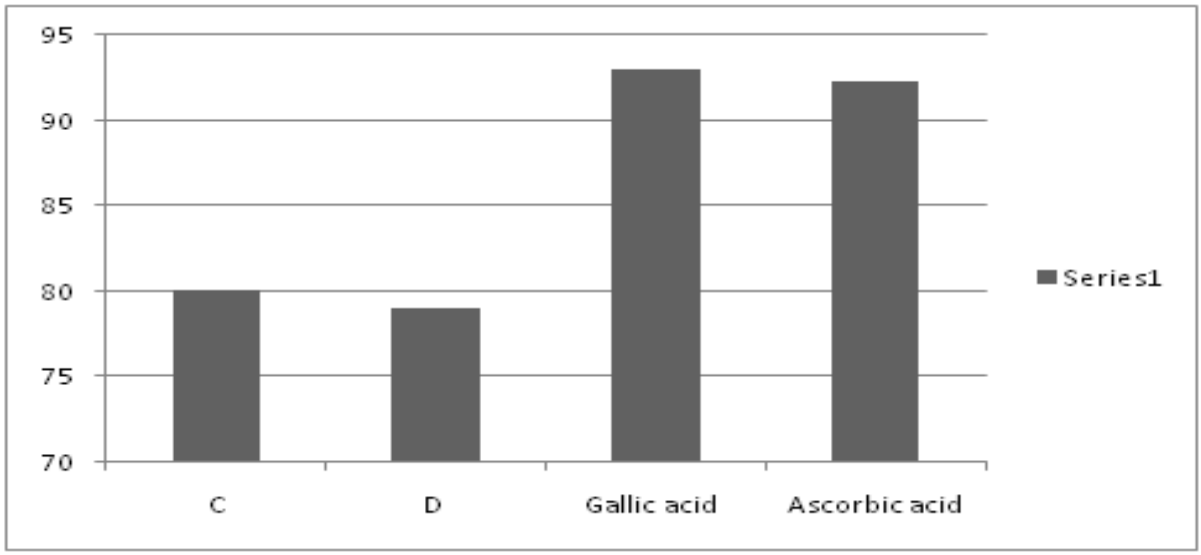

Fig. 2. % of RSA of compound C, D and the standards.

UV and IR spectra of "D"

The UV spectra confirm anthraquinones skeleton as in compound **C.** The absorptions at (1701–1728) and (1590) in cm^{-1} of the IR spectrum showed the presence of ester group and conjugated carbonyl groups of anthraquinone nucleus but a peak at 1715 cm^{-1} was found to be disappear which showed the absence of non conjugated carbonyl group in compound D. An additional strong absorption at 1270--1230 cm^{-1} showed the ether functionality.

The molecular formula was determined by HREI-MS as $C_{25}H_{26}O_6$ (m/z =422.1729; calcd.422.1719), which shows 10 degrees of unsaturation. Eight of them are eventually accounted for anthraquinone ring, one for ketonic and last one for ester carbonyls group.

^{1}H-NMR and ^{13}C-NMR spectra of compound "D"

The^{1}H-NMR and ^{13}C-NMR spectra of compound "D" (Table 1) was very similar to that of "C", except for the presence of additional peaks due to ether group(H-14=3.79s, 56.60=C-14) plus absence of carbonyl group peaks (H-14=3.91s, H-16=2.13s,C-14=48.40,C-15=206.01,C-16=31.30 of compound).^{1}H-NMR and ^{13}C-NMR spectra and molecular formula determined by HREI-MS showed that CH_3O group is present in compound D in place $-CH_2$-CO-CH_3 group of compound C.

RSA of compound C & D

The results of Table2 indicate significant radical scavenging activity C & D. Both the compounds have round about same RSA. Both of the isolated compounds have less radical scavenging property than ascorbic and gallic acid. Both the compounds are slightly less active than anthraquinones reported by Khabir *et al.* (Khabir, A *et al.* 2017) %DPPH inhibition has also been shown graphically (Fig. 1).

Conclusion

The phytochemical investigation of the medicinal plant *Rumex obtusifolius* were carried out by using various chemical, chromatographic and spectroscopic techniques. Two new compounds were isolated from the DCM soluble fraction, both of which have significant antioxidant activities. *R. obtusifolius* is a common ingredient of traditional and medicine in some countries ofthe world, so, further investigations on this plant are recommended to exploit its hiddenmedicinal values and importance.

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