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BIOACTIVITY OF *RUMEX OBTUSIFOLIUS* (POLYGONACEAE)

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Abstract –*Rumex obtusifolius* L. (Polygonaceae), commonly known as 'broad-leaf dock', is one of the most common Irish wayside weeds, and it also occurs in silage fields, on river banks, in ditches and on waste grounds. The ethnobotanical uses of this species include its use as an antidote to nettle, depurative, astringent, laxative, and tonic, and in the treatment of sores, blisters, burns, cancer and tumors. The bioactivities of *n*-hexane, dichloromethane (DCM) and methanol (MeOH) extracts of the leaves of *R. obtusifolius* were assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, the newly developed micro-titer-based antimicrobial assay incorporating resazurin as an indicator of cell growth, and the brine shrimp lethality assay. The most potent free radical scavenging activity was displayed by the MeOH extract with a RC_{50} value of 7.80 x 10^{-2} mg/mL. Among the fractions obtained from solid-phase extraction (SPE) of the MeOH extract, the 50% aqueous methanolic SPE fraction exhibited the highest levels of free radical scavenging property (RC_{50} = 1.05 x 10^{-2} mg/mL). While the *n*-hexane extract did not show any antibacterial activity at test concentrations, the DCM extract was active only against *Escherichia coli*. However, the MeOH extract as well as the 50% and 80% SPE fractions of the MeOH extract showed significant antibacterial property against all bacterial strains tested. None of the extracts or fractions exhibited any significant toxicity towards brine shrimps.

Keywords: Rumex obtusifolius, Polygonaceae, antioxidant, antibacterial, DPPH, brine shrimp lethality

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INTRODUCTION

Rumex obtusifolius L. (Polygonaceae), commonly called 'broad-leaf dock', is one of the most common Irish wayside weeds, and it also occurs in silage fields, on river banks, in ditches and on waste grounds. It is native to Northern Ireland and a number of other countries in Africa, temperate Asia, and Europe (GRIN Taxonomy Database, 2009). This perennial plant has long been used in folklore medicine. The ethnobotanical uses of this species include its use as an antidote to nettle, depurative, astringent, laxative, and tonic, and in the treatment of sores, blisters, burns, cancer and tumors (Dr Duke's Phytochemical and Ethnobotanical Databases, 2009). Previous studies of this plant revealed the presence of anthracene derivatives, flavonoids, procyanidins and oxalic acid (Trichopoulou et al., 2000; Spencer et al., 2007; Wegiera et al., 2007). We now report on the free radical scavenging (antioxidant) and antibacterial activities, and the brine shrimp lethality of *n*-hexane, dichloromethane (DCM) and methanol (MeOH) extracts of the leaves of *R. obtusifolius* using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Takao et al., 1994; Kumarasamy et al., 2002; 2007), the newly developed micro-titer-based antimicrobial assay incorporating resazurin as an indicator of cell growth (Sarker et al., 2007), and the brine shrimp lethality assay (Meyer et al., 1982), respectively.

MATERIALS AND METHODS

Plant Materials

The leaves of *Rumex obtusifolius* L. were collected from Aghadowey, Coleraine, Northern Ireland, during May-June 2007. A voucher specimen (2007001_SS) for this collection has been retained in the herbarium of the School of Biomedical Sciences, University of Ulster, UK.

Extraction of plant materials

The dried and ground leaves of *R. obtusifolius* (35.5 g) were Soxhlet-extracted sequentially with 250 mL of *n*-hexane, dichloromethane (DCM) and methanol (MeOH). Filtered extracts were dried using a rotary evaporator at 45° C.

Solid-phase extraction (SPE): fractionation of the MeOH extract

The MeOH extract was further fractionated on a Sep-Pak C_{18} reversed-phase cartridge (10 g) under vacuum using a MeOH-water solvent mixture of decreasing polarity, e.g. 20%, 50%, 80% and 100% MeOH-water, to obtain four SPE fractions. Fractions were dried using a rotary evaporator at 45° C.

Free-radical-scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin and Trolox® were obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao et al., (1994) was adopted with suitable modifications (Kumarasamy et al., 2002). DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 µg/mL.

Qualitative assay: Test sample solutions were applied on a TLC plate and sprayed with DPPH solution using an atomizer. It was allowed to develop for 30 min. The color changes (purple on white) were noted.

Quantitative assay

The extracts/fractions were dissolved in MeOH to obtain the test concentration 10 mg/mL. Dilutions were made to obtain concentrations of $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$ $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to take place. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive controls, quercetin and Trolox® (1 mg/mL in MeOH).

Antibacterial assay

The antibacterial activity of the extracts were assessed against six bacterial strains, *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (NCTC 10400), *Escherichia coli* (ATCC 8739), ampicillin-resistant *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 1803) and *Salmonella typhii* (NCTC 10203), obtained from the Biotechnology Laboratory, School of Biomedical Sciences, University of Ulster.

Disc diffusion assay

The conventional disc diffusion method (Bauer et al, 1966; Cruickshank, 1968) was employed for the initial assessment of antibacterial potential of the extracts. Sterile 6.0 mm diameter blank discs (BBL, Cocksville, USA) were impregnated with test substances at a dose of 500 µg/disc. These discs, along with the positive control discs (ciprofloxacin, 10 µg/disc) and negative control discs were placed on Petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates were kept in an incubator (37°C) to allow the growth of the bacteria. The antibacterial activities of the test agents were determined by measuring the diameter of the zone of inhibition in terms of millimeter.

Table 1. Antioxidant activities and brine shrimp toxicity of the extracts and fractions of *Rumex obtusifolius*

Extracts / Fractions	Antioxidant activity[a]		Brine shrimp toxicity (LD_{50} in mg/mL)
	Qualitative	Quantitative (RC_{50} in mg/mL)	
n-Hexane	+	-	NP
DCM	+	-	1.00
MeOH	+	7.80 x 10-2	>1.00
SPE 20%b	+	18.8 x 10-1	>1.00
SPE 50%b	+	1.05 x 10-2	>1.00
SPE 80%b	+	26.5 x 10-1	>1.00
SPE 100%b	+	-	>1.00
Trolox®	+	2.60 x 10-3	NP
Podophylotoxin	NP	NP	2.79 x 10-3

[a]Determined by the DPPH assay.
= No activity detected at test concentrations; + = Activity; NP = Not performed
[b]Fractions obtained from solid-phase extraction of the MeOH extract by a mixture of MeOH-water of the specified proportions.

Resazurin micro-titre assay

The recently published 96-well micro-titer assay using resazurin as the indicator of cell growth (Sarker et al., 2007) was employed for the determination of the minimum inhibitory concentration (MIC) of the active extracts.

Assessment of bacteriostatic/bactericidal property: The agar plate was seeded with the mixture from the well which was just before the well of the MIC, using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates were kept in an incubator (37°C) to allow the growth of the bacteria. Any bacterial growth would indicate the bacteriostatic property of the extract, and no growth would be an indicator of bactericidal activity (Granger et al., 2009).

Brine Shrimp Lethality (BSL) assay

Brine shrimp eggs were purchased from Water Life, Middlesex, UK. The bioassay was conducted following the procedure published previously (Meyer et al., 1982). LD_{50}s were determined from the 24 h counts using the Probit analysis method (Finney, 1971). Percentage mortalities were adjusted relative to the natural mortality rate of the control, following Abbots formula P = (Pi – C)/(1 – C), where P denotes the observed nonzero mortality rate and C represents the mortality rate of the control.

RESULTS

The free radical scavenging (antioxidant) and antibacterial activities, and brine shrimp lethality of *n*-hexane, DCM and MeOH extracts of the leaves of *Rumex obtusifolius* were evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (Takao et al., 1994; Kumarasamy et al., 2002; 2007), the micro-titer-based antimicrobial assay incorporating resazurin as an indicator of cell growth (Sarker et al., 2007), and the brine shrimp lethality assay (Meyer et al., 1982), respectively. In the TLC-based qualitative antioxidant assay using the DPPH spray all extracts and fractions showed antioxidant properties indicated by the presence of a yellow/white spot on a purple background on the TLC plates. However, in the quantitative assay, the *n*-hexane and the DCM extracts did not show any detectable activity at test concentrations. The RC_{50} value of the MeOH extract was 7.80 x 10^{-2} mg/mL. Among the SPE fractions of the MeOH extract, the 50% aqueous methanolic

Table 2. Antibacterial activity of the extracts and fractions of *Rumex obtusifolius*

Extracts / Fractions	Antibacterial activity											
	Disc diffusion assay (in mm)						Resazurin assay (MIC = mg/mL)					
	BC	BS	EC	AEC	SA	ST	BC	BS	EC	AEC	SA	ST
n-Hexane	-	-	-	-	-	-	-	-	-	-	-	-
DCM	-	-	10	-	-	-	-	-	12.5	-		
MeOH	14	10	15	20	8	12	3.12	3.12	3.12	1.56	25.0	12.5
SPE 20%[a]	-	-	-	-	-	-	-	-	-	-	-	-
SPE 50%[a]	15	12	18	22	10	14	3.12	3.12	3.12	1.56	12.5	12.5
SPE 80%[a]	8	8	12	16	8	8	3.12	25.0	25.0	6.25	25.0	25.0
SPE 100%[a]	-	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	32	30	30	30	30	30	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-7}	2.5×10^{-6}	2.5×10^{-8}	2.5×10^{-7}

= No activity detected at test concentrations
[a]Fractions obtained from solid-phase extraction of the MeOH extract by a mixture of MeOH-water of the specified proportions.
BC = *Bacillus cereus* (ATCC 11778), BS = *Bacillus subtilis* (NCTC 10400), EC = *Escherichia coli* (ATCC 8739), AEC = Ampicillin-resistant *Escherichia coli* (NCTC 10418), SA = *Staphylococcus aureus* (NCTC 1803) and ST = *Salmonella typhii* (NCTC 10203).

fraction displayed the highest level of antioxidant property (RC_{50} = 1.05×10^{-2} mg/mL) (Table 1). In the antimicrobial assays, the DCM extract was active only against *Escherichia coli*, and the MeOH extract as well as the 50% and 80% Sep-Pak fractions of the MeOH extract showed significant antibacterial property against all bacterial strains tested (Table 2). None of the extracts showed any significant toxicity towards brine shrimps (LD_{50} = >1.00 mg/mL) (Table 1).

DISCUSSION AND CONCLUSION

The DPPH antioxidant assay is based on the principle that 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, is decolorized in the presence of free radical scavengers (antioxidants). The odd electron in the DPPH radical is responsible for the absorbance at 517 nm, and also for visible deep purple color (Kumarasamy et al., 2002). When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. In the TLC-based qualitative antioxidant assay using the DPPH spray all extracts and SPE fractions showed antioxidant properties indicated by the presence of a yellow/white spot on a purple background on the TLC plates. However, in the quantitative assay, the *n*-hexane and the DCM extracts did not show any detectable activity at test concentrations. The RC_{50} value of the MeOH extract was 7.80×10^{-2} mg/mL. Among the SPE fractions, the 50% aqueous methanolic fraction displayed the most potent free radical scavenging (antioxidant) property (RC_{50} = 1.05×10^{-2} mg/mL). The DPPH scavenging capacity of the extracts was compared with the known antioxidant, Trolox®. From the results it was obvious that the antioxidant properties of this plant were owing to medium polarity compounds. As anthracene derivatives, flavonoids, procyanidins and oxalic acid were previously reported from this plant (Trichopoulou et al., 2000; Spencer et al., 2007; Wegiera et al., 2007), those compounds might be responsible for the antioxidant activity of this plant. Most of these compounds are polyphenolic compounds, which are known to possess such activities because of the

presence of phenolic hydroxyl groups. The DPPH radical scavenging activities of the positive control as well as the test extracts are presented in Table 1.

The conventional disc diffusion method (Bauer et al, 1966; Cruickshank, 1968) was applied to assess the antibacterial property of *n*-hexane, DCM and MeOH extracts of the leaves of *R. obtusifolius*, and the SPE fractions obtained from the MeOH extract (Table 2). The *n*-hexane extract did not show any antibacterial activity at test concentrations, the DCM extract was active only against *Escherichia coli*, and the MeOH extract was active against all strains of bacteria tested. Among the SPE fractions, while the 20% and 100% fractions were totally inactive, the 50% and 80% fractions were active against all test organisms. The most notable antibacterial activities were observed with the MeOH extract and the 50% Sep-Pak fraction against ampicillin-resistant *E. coli*, with zones of inhibition of 20 and 22 mm, respectively.

The MIC values of the extracts were determined by the newly developed resazurin micro-titer assay (Sarker et al., 2007). The MeOH extract was the most potent (MIC range 1.56–25.0 mg/mL) among the extracts, and was most active against ampicillin-resistant *E. coli* (MIC = 1.56 mg/mL) (Table 2). Similar activities were displayed by the 50% and the 80% SPE fractions. The active extracts/fractions were found to be bacteriostatic rather than bactericidal. The antibacterial activity of *R. obtusifolius* was mainly due to medium polarity compounds, e.g. phenolics, present in the polar MeOH extract, and in the two middle fractions, the 50% and the 80%, of the SPE fractions.

The brine shrimp lethality assay (BSL) has been used routinely in the primary screening of the crude extracts as well as the isolated compounds to assess the toxicity towards brine shrimps, which could also provide an indication of possible cytotoxic properties of the test materials (Meyer et al., 1982). It has been established that the cytotoxic compounds usually show good activity in the BSL assay, and this assay can be recommended as a guide for the detection of antitumor and pesticidal compounds because of its simplicity and cost-effectiveness. The extracts of *R. obtusifolius* did not show any significant toxicity towards brine shrimps in the BSL assay (Table 1). Owing to a high degree of lipophilicity, the *n*-hexane extracts could not be tested. While the LD_{50} values of the DCM and the MeOH extracts were, respectively, 1.00 mg/mL and >1.00 mg/mL, that of the Sep-Pak fractions were >1.00 mg/mL, indicating that *R. obtusifolius* had generally low levels of toxicity towards brine shrimps. The LD_{50} value of the positive control, podophylotoxin, was 2.80×10^{-3} mg/mL.

The presented findings could provide some possible scientific basis for the ethnobotanical uses of this plant, particularly its use for the treatment of sores, blisters, burns, cancer and tumors. As none of the extracts or fractions was particularly toxic to brine shrimps, indicating a low level of toxicity, this plant might be used as a source of less toxic but potent antioxidant and antibacterial agents.

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