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ANIMAL SCIENCE

In vitro antimicrobial activity of material from 450 European plant species towards *Clostridium perfringens*, enterotoxigenic *Escherichia coli* (ETEC) and *Campylobacter jejuni*.

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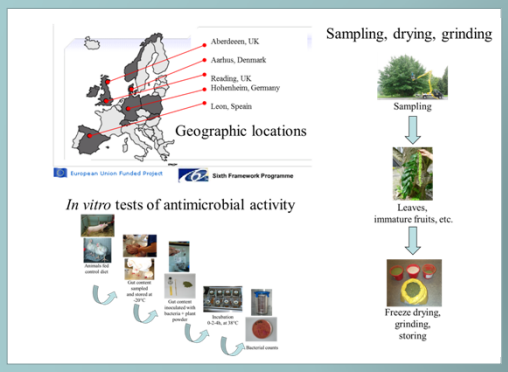
Introduction and Aim

To identify potential alternatives to in-feed antibiotics, the EU FP6 project REPLACE (Food-CT-2004-506487) screened 450 European plant species (representing 100 families) for antimicrobial activity.

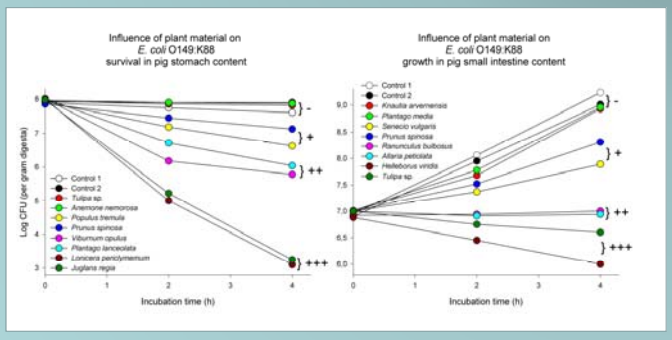
Materials and Methods

The plant material (e.g. leaves, flowers, fruits), collected at five geographical locations in Europe, was dried, ground and stored at room temperature in dark. No extraction of potential active compounds was involved. The antimicrobial activity towards *C. perfringens* type A strain 99.63206-34 was tested in chicken ileum extract medium (pH 6.5) and *C. jejuni* DSM 4688^T was tested in brain heart infusion broth (pH 7.0). The activity towards the ETEC strain *E. coli* O149:K88 was tested in two *in vitro* assays; survival in pig stomach content (pH 4.2-4.5) and growth in pig small intestinal content (pH 6.5-7.0).



Results

The inhibitory levels were arbitrarily defined as high (+++), medium (++), low (+) or insignificant (-) as illustrated below for the *E. coli* assays **(Fig. 1)**.



Clostridium perfringens was in general most prone to inhibition, and 81 samples, particular several members of the families *Cistaceae*, *Ericaceae*, *Fagaceae* and *Rosaceae*, inhibited growth completely with an inclusion level of 5% **(Table 1)**, and 31 of these showed the same result with an inclusion level of 1% **(Table 2)**. Species like *Eugenia caryophyllata* and *Quercus robur* showed significant inhibition even at 0.1% (data not shown).

The ETEC strain was particularly inhibited by members of the families *Juglandaceae*, when tested in stomach content, and members of *Brassicaceae*, *Juglandaceae*, *Liliaceae* and *Ranunculaceae* in small intestinal content **(Table 1)**. For further details on plant species see attached **Table 3 (hand-out)**.

Campylobacter jejuni was inhibited most significantly by members of *Fagaceae*, *Juglandaceae* and *Liliaceae* **(Table 1)**.

Table 1. Antimicrobial activity towards *Clostridium perfringens* strain 99.63206-34, *E. coli* O149:K88 and *Campylobacter jejuni* DSM 4688[T] of plant material (5% inclusion rate) by species of different plant families. Approx. 250 of the plant samples showed no inhibitory effect against either of the tested bacteria, whereas the remaining approx. 200 plant samples showed some level of antibacterial activity.

Plant family	Plant species per family[a]	Inhibition level[b] towards *C. perfringens* +++	++	+	-	*E. coli* (stomach) +++	++	+	-	*E. coli* (small intestine) +++	++	+	-	*C. jejuni* +++	++	+	-
Apiaceae	21			2	19				21			1	20				1
Araceae	2				2			1	1				2				
Araliaceae	2			1	1				2			1	1				1
Asteraceae	54		1	12	41		1		53		4	4	46			5	2
Berberidaceae	4	1		1	2			1	3			1	3				1
Betulaceae	3	2	1						3				3				
Bignoniaceae	2	1	1				2				1		1		1		
Boraginaceae	7			1	6		1		6			2	5			2	
Brassicaceae	9				9			1	8	2			7	1	1		
Campanulaceae	3			1	2				3			1	2				1
Caprifoliaceae	10		2	1	7	1	2		7		1	3	6	1	1	1	1
Caryophyllaceae	6				6				6				6				
Celastraceae	2	2							2		1		1		1		
Chenopodiaceae	5				5				5		1	1	3			2	
Cistaceae	7	4	3						7		1	1	5				2
Convolvulaceae	2				2				2				2				
Crassulaceae	3				3				3		2		1				1
Cucurbitaceae	4				4				4				4				
Cupressaceae	5	4			1				5				5				
Dipsacaceae	3				3				3			1	2	1			
Dryopteridaceae	2	2							2				2				
Equisetaceae	2				2				2				2				
Ericaceae	10	6	3		1		2	2	6				10				
Euphorbiaceae	6		1	2	3			1	5		1		5				1
Fabaceae	24	1		2	21			1	23		2		22			1	1
Fagaceae	7	6	1			2			5		1	1	5	2			1
Hippocastanaceae	2	2							2				2				1
Hydrangeaceae	2		1		1			1	1			1	1				1
Juglandaceae	3	1	1	1		3				1	1	1		2			1
Juncaceae	2				2				2				2				
Lamiaceae	37	1	2	9	25			7	30		5	4	28		3	4	3
Lauraceae	4	1	1		2		1	1	2		1	1	2		2		
Liliaceae	9			2	7			2	7	2	2		5	3	1		
Linaceae	2				2				2		1	1			1	1	
Magnoliaceae	2				2				2			1	1			1	
Malvaceae	4				4				4				4				
Myrtaceae	3	3				1			2	1	1		1	1			1
Oleaceae	10	1		4	5			1	9			1	9			1	
Onagraceae	3	3							3				3				
Paeoniaceae	2	1			1				2				2				
Papaveraceae	3			3					3				3				
Pinaceae	5	1		3	1			3	2		2		3				2
Plantaginaceae	3		1		2		1		2				3				
Poaceae	5				5				5				5				
Polygonaceae	9	3	2	2	1		2	1	6		1	1	7			1	1
Primulaceae	2		1		1				2				2				
Ranunculaceae	15	1	1	2	11		1	1	13	4	1		10	2	1	2	1
Resedaceae	3			2	1			2	1			3	3				
Rosaceae	28	16	3	1	8		1	1	26		2	3	23		2	1	1
Rubiaceae	3			2	1		1		2		1		2				
Rutaceae	4		1		3			1	3			1	3				1
Salicaceae	5	2	1	2				2	3		1	1	3				1
Saxifragaceae	2	2				1			1		1		1			1	
Scrophulariaceae	5		2	1	2	1			4		1		4	1			
Solanaceae	6				6				6		1		5				1
Theaceae	3			3					3		1		2				1
Tiliaceae	2	2					1		1				2				
Valerianaceae	2	2					1	1			1		1	1			
Verbanaceae	2	1		1			1		1				2				
Violaceae	2		1	1					2				2				
Zingiberaceae	3			1	2				3				3				
Families with one s	48	9	7	5	26		5	8	32	1	3	2	39	1	2	3	2
	447	81	38	68	260	9	23	39	376	11	42	35	359	16	16	27	30
		447				447				447				89			

Table 2. Influence of plant material[a] (1% w/w) from selected plant species[b] on growth of *Clostridium perfringens* strain 99.63206-34 in chicken ileum extract medium.The plant species included all showed high inhibitory effect towards *C. perfringens* when added at a koncentration of 5% (w/w) and were then further investigated with an inlcusion rate of 1% (w/w).

Plant taxa		Inhibition level[c]	Plant taxa		Inhibition level
Family	Species		Family	Species	
Berberidaceae	*Podophyllum hexandrum* (L. S)[d]	+	*Myrtaceae*	*Eucalyptus globulus* (L)	++
Betulaceae	*Betula pendula* (L)	++		*Eugenia caryophyllata* (B)	+++
Bignoniaceae	*Catalpa speciosa* (L)	++		*Pimenta officinalis* (F)	+
Celastraceae	*Euonymus alatus* (L. S)	++	*Oleaceae*	*Ligustrum vulgare* (L. S)	+
Cistaceae	*Cistus ladanifer* (L. S)	+	*Onagraceae*	*Chamerion augustifolium* (L. S. F)	+++
	Cistus laurifolius (L. S)	+++		*Epilobium hirsutum* (WO)	++
	Halimium lasianthum (L. S. Fl)	+++		*Oenothera biennis* (WO)	+++
	Xolantha guttata (L. S. Fl)	+++	*Paeoniaceae*	*Paeonia officinalis* (L. S)	+++
Cupressaceae	*Juniperus thurifera* (L. S)	++	*Pinaceae*	*Abies nordmannia* (L)	++
	Metasequoia glyptostroboides (L)	+++	*Platanaceae*	*Platanus X hybrida* (L)	+++
	Sequoiadendron giganteum (L)	++	*Polygonaceae*	*Polygonum japonicum* (L. S)	++
	Thuja plicata (L)	++		*Polygonum aviculture* (L)	-
Dennstaedtiaceae	*Pteridium aquilinum* (L)	+++		*Rheum nobile* (L. S)	-
Dryopteridaceae	*Athyrium filix-femina* (L. S)	++	*Punicaceae*	*Punica granatum*	+++
	Dryopteris filix-mas (L. S)	++	*Ribesiaceae*	*Ribes nigrum* (L)	++
Ericaceae	*Arctostaphylos uva-ursi* (L. S)	+	*Rosaceae*	*Alchemilla alpina* (L. S)	+++
	Erica arborea (WO)	++		*Alchemilla vulgaris* (L)	+++
	Gaultheria procumbens (L. S)	+++		*Alchemilla xanthochlora* (L. S. F)	++
	Rhododendron ponticum (L)	-		*Filipendula ulmaria* (WP)	++
	Vaccinium myrtillus (L. S)	++		*Filipendula vulgaris* (L. S)	-
	Vaccinium vitis-idaea (L. S)	+++		*Geum urbanum* (L. S)	++
Fabaceae	*Gleditsia japonica* (L)	-		*Potentilla anserina* (WO)	+++
	Robinia pseudoacacia (L)	-		*Potentilla aurea* (L)	++
Fagaceae	*Castanea sativa* (L)	+++		*Potentilla reptans* (L. S. F)	+++
	Castanea sativa (IFr)	+++		*Rosa canina* (L. S. F)	+++
	Castanea sativa (MFr)	-		*Rosa gallica* (L. S)	++
	Fagus sylvatica (L. S)	+++		*Rosa rubiginosa* (L. S)	+++
	Quercus robur (L)	+++		*Rubus fruticosus* (L. S)	+++
	Quercus robur (IF)	+++		*Rubus idaeus* (L)	++
	Quercus ilex (L)	++		*Rubus ulmifolius* (L. S)	+++
Hamamelidaceae	*Parrotia persica* (L)	+++		*Sanguisorba minor* (L. S. F)	+++
Hippocastanaceae	*Aesculus hippocastanum* (IFr)	++	*Salicaceae*	*Salix fragilis* (L)	+
	Aesculus hippocastanum (L)	+		*Salix viminalis* (L)	+
Juglandaceae	*Pterocarya fraxinifolia* (L. IFr)	++	*Saxifragaceae*	*Bergenia cordifolia* (L. S)	++
	Juglans regia (L)	-		*Peltiphyllum peltatum* (L. S)	+++
Lamiaceae	*Salvia apiana* (L)	+++	*Taxaceae*	*Taxus baccata* (L)	-
Lauraceae	*Cinnamomum cassia* (L)	++	*Tiliaceae*	*Tilia cordata* (L. F)	+
	Laurus nobilis (L)	-		*Tilia europea* (L. S)	+
Lythraceae	*Lythrum salicaria* (WO)	+++	*Valerianaceae*	*Valeriana officinalis* (WP)	++
Myricaceae	*Myrica gale* (L. S)	+	*Verbenaceae*	*Vitex agnus-castus* (L. S)	+

[a]The plant material was added to the assays as freeze dried powder in a concentration of 1% (weight/weight). For further information on the specific plant fraction used in each case see complete plant collection list (http://www.rowett.ac.uk/rumen_up/RumenUp_Plants.xls)

[b]The plant species included all showed high inhibitory effect towards *C. perfringens* when added at a koncentration of 5% (w/w) and were then further investigated with an inlcusion rate of 1% (w/w).

[c]The inhibition levels were defined as +++: High; ++: Medium; +: Low; -: Insignificant

[d]The fractions used were B: buds; Fl: flowers; Fr: fruits; IFr: immature fruites; L: leaves; MFr: mature fruits; S: stems; WO: whole overground; WP: whole plant

Conclusion

The present study demonstrates that significant levels of antimicrobial activity can be found in non-extracted material from a wide range of European plant species; the active compounds and their potential as alternative to antibiotic growth promoters in animal production is presently under scrutiny.

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