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The pharmacological actitvity of Rhoicissus tridentata subsp cuneifolia in relation to parturition

Katsoulis, Lynn Coleen

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Autumn leaves

Ach conc	52.2.e	53.1.i	54.1.b	55.1.e	57.2.l	57.4.l	58.2.e	58.4.e	60.1.b	60.4.b - B	81.1.h - K	81.3.h	82.2.e	82.4.e	83.2.b
-9.5	3.0	0.0	37.7	28.1	10.2	6.6	75.1	3.8	18.5	14.4	0.0	7.2	83.2	32.9	43.3
-9	3.0	0.0	31.5	0.0	8.2	0.0	33.2	0.0	13.0	14.4	0.0	1.6	71.5	32.9	26.7
-8.5	3.0	0.0	31.5	20.2	8.2	0.0	33.2	0.0	13.0	14.4	0.0	1.6	71.5	32.9	26.7
-8	3.0	0.0	31.5	0.0	8.2	0.0	33.2	0.0	13.0	14.4	0.0	1.6	71.5	32.9	26.7
-7.5	3.6	0.0	31.5	0.0	8.2	0.0	33.2	0.0	13.0	14.4	0.0	1.6	71.5	32.9	26.7
-7	3.6	0.0	34.2	0.0	8.2	0.0	33.2	6.3	13.0	14.4	5.0	9.6	71.5	32.9	56.0
-6.5	3.6	0.0	34.2	15.7	12.2	14.2	33.2	7.5	20.7	16.1	5.6	12.8	71.5	43.2	66.7
-6	3.6	7.2	34.2	27.0	16.3	17.0	33.2	13.6	32.6	16.1	17.9	36.0	76.5	65.1	78.7
-5.5	4.2	7.2	35.6	27.5	18.4	23.6	33.2	15.0	43.5	23.7	33.0	57.6	84.9	82.2	86.7
-5	8.2	30.4	38.4	41.6	28.6	30.2	38.4	25.0	65.2	40.3	58.1	76.8	84.9	91.8	94.7
-4.5	10.6	39.1	38.4	47.8	38.8	44.3	42.8	32.5	80.4	52.5	73.7	87.2	86.0	94.5	98.7
-4	16.4	53.6	45.2	52.8	65.3	68.9	48.0	46.3	92.4	75.4	86.6	94.4	98.3	97.3	102.0
-3.5	24.2	71.0	49.3	60.7	73.5	77.4	50.7	55.0	96.7	85.2	93.9	98.0	98.3	98.6	102.0
-3	35.2	97.1	63.0	64.0	81.6	83.0	50.7	58.8	98.9	92.8	97.8	99.2	101.7	97.9	102.7
-2.5	43.9	100.0	65.8	58.4	79.6	94.3	46.3	58.8	100.0	94.1	98.9	100.0	101.7	108.2	100.0

Autumn leaves - continued

Ach conc	83.4.b - K	84.2.e - L	84.4.e	85.1.b	85.3.b	86.1.e	86.3.e
-9.5	3.9	36.0	15.2	30.0	52.9	22.7	3.3
-9.0	3.9	36.0	13.6	30.0	52.9	22.7	0.0
-8.5	3.9	36.0	13.6	30.0	52.9	22.7	0.0
-8.0	3.9	36.0	13.6	30.0	52.9	22.7	0.0
-7.5	3.9	36.0	13.6	30.0	52.9	22.7	0.0
-7.0	13.4	50.6	13.6	30.0	52.9	22.7	4.6
-6.5	22.3	57.3	20.9	30.0	54.9	22.7	11.1
-6.0	62.6	78.7	45.0	30.8	61.3	36.0	29.5
-5.5	76.5	87.6	66.0	33.1	76.5	49.3	46.6
-5.0	92.7	93.3	84.8	57.7	91.2	65.3	68.9
-4.5	98.3	97.8	90.1	66.9	102.9	76.0	79.3
-4.0	102.2	101.1	96.9	87.7	110.8	84.0	88.5
-3.5	105.0	102.8	98.4	98.5	112.7	86.7	92.5
-3.0	105.0	103.4	97.4	104.6	112.7	89.3	95.1
-2.5	105.0	102.2	97.4	106.2	113.7	89.3	95.1

Fruit

	97.3.h	98.2.e	99.1.b	100.1.b
-9.5	11.0	28.2	33.6	57.8
-9.0	11.0	28.2	33.6	57.8
-8.5	11.0	28.2	33.6	57.8
-8.0	11.0	28.2	33.6	57.8
-7.5	11.0	28.2	33.6	57.8
-7.0	11.0	28.2	37.0	59.3
-6.5	12.2	28.2	42.9	65.2
-6.0	17.1	33.1	58.8	68.1
-5.5	26.8	45.1	63.4	75.9
-5.0	39.0	58.5	79.8	83.7
-4.5	53.7	70.4	89.1	94.1
-4.0	64.6	78.9	96.6	100.0
-3.5	70.7	82.4	102.1	101.5
-3.0	74.4	83.8	102.1	103.7
-2.5	74.4	83.8	102.1	103.7

Activity of plant extract alone - data for box and whisker plots

Roots																					
Spring	9.7	0.0	4.3	14.2	0.0	0.0	4.0	4.4	0.0	0.0	45.3	8.4	7.0	6.5							
Summer	27.5	20.4	14.1	46.4	7.6	26.2	8.3	41.5	85.0	25.9	30.6	6.6	75.8	15.6							
Autumn	3.7	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	6.9	15.2	5.7	4.7								
Winter	37.8	5.2	78.4	12.9	17.8	11.8	0.0	25.0	25.0	1.6	19.6	0.0	29.2	18.8	20.2	17.1	5.2	50.0	15.9		
Tubers																					
Spring	0.0	0.0	7.3	11.3	26.1	38.4															
Summer	21.5	44.4	68.7	56.0	77.9	30.8															
Autumn	71.0	61.3	33.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	77.9	30.8									
Winter	25.0	0.0	0.0	4.5	61.4	2.3	0.0	10.4	15.8	0.0	24.0	0.0	8.0	15.4	45.5	9.3	7.3	10.9			
Stems																					
Spring	9.9	8.1	10.2	5.7	41.5	20.7	13.8	0.0	6.6	11.3	41.8	15.4	17.6	20.0	0.0						
Summer	46.7	39.8	19.8	12.2	81.9	34.1	28.1	44.2	29.9	53.3	25.9	46.7									
Autumn	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7	5.9	86.3	43.5			
Winter	23.8	35.4	12.4	88.2	78.0	27.5	6.6	32.1	20.0	30.3	7.2	24.7	15.2	14.6	10.5	0.0	0.0	53.2	31.9		
Leaves																					
Summer	8.5	7.8	0.0	8.2	10.2	12.2	55.6	7.7	56.8	11.6	9.4	10.0	55.8	28.2							
Autumn	3.0	0.0	37.7	28.1	10.2	6.6	75.1	3.8	18.5	14.4	0.0	7.2	83.2	32.9	43.3	3.9	36.0	15.2	30.0	53.9	22.7	3.3
Fruit	11.0	28.2	33.6	57.8	16.3																

Stability of *Rhoicissus*

Acetylcholine standard

Ach conc	81.1.a	81.1.d	81.3.a	81.3.d	82.1.g	82.3.g	83.2.d	83.2.g	83.4.d	83.4.g	116.1.aM2	117.2.a	118.2.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	7.8	0.0	0.0	4.1	22.8	9.9	6.4	1.4	0.0
-6.5	0.0	3.7	3.1	9.9	12.6	8.8	58.8	0.0	38.0	14.3	10.0	11.5	24.0
-6	2.4	10.8	13.0	27.4	24.3	26.3	77.1	56.6	66.3	58.2	22.7	41.0	56.9
-5.5	9.6	26.2	34.4	46.4	42.7	49.8	81.0	75.9	78.3	76.4	40.9	64.7	79.0
-5	28.7	48.7	59.5	69.2	57.3	70.2	88.6	86.9	88.0	87.9	63.6	84.2	89.8
-4.5	45.5	69.3	75.6	80.6	80.6	84.9	93.5	92.4	94.0	93.4	76.4	93.5	95.2
-4	71.3	84.7	87.0	91.3	90.3	94.1	98.0	97.2	97.8	97.8	90.9	97.8	98.8
-3.5	84.4	92.1	96.2	95.8	97.1	98.5	99.3	99.3	100.0	100.0	96.4	100.0	100.0
-3	96.4	97.9	97.7	99.6	100.0	100.0	100.0	100.0	100.0	100.0	99.1	100.0	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Acetylcholine standard - continued

Ach conc	118.4.c	127.1.c	133.2.dK2t	134.4.d	136.2.g	136.4.g	133.3.dK2t	134.3.d	136.2.d	136.4.d	133.1.dL2t	134.1.d
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.0	27.7	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4
-6.5	42.2	3.7	15.1	7.6	27.6	0.0	0.0	9.3	34.3	0.0	0.0	15.9
-6.0	56.6	6.3	24.6	31.3	41.4	29.1	2.8	22.5	44.0	21.0	5.3	22.0
-5.5	72.3	17.4	46.0	55.0	68.3	55.7	26.8	49.7	69.7	65.4	21.3	42.7
-5.0	85.5	36.9	73.0	75.6	81.4	74.7	53.5	72.2	86.9	79.0	41.3	58.5
-4.5	96.4	65.3	84.1	87.0	90.3	83.5	71.8	88.7	93.1	91.4	60.0	67.1
-4.0	97.6	91.7	100.0	95.4	95.2	93.7	94.4	97.4	97.1	98.8	78.7	82.9
-3.5	100.0	95.8	100.0	100.0	100.0	97.5	97.2	100.0	100.0	100.0	90.7	92.7
-3.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	97.6
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Acetylcholine standard - continued

Ach conc	135.2.j	135.4.j	136.1.g	136.3.g	81.1&2K1	81.3&4	82.1&2	82.3&4	83.1&2	83.3&4
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.0	0.0	0.0	23.7	0.0	8.4	5.2	9.3	9.1	4.7	10.1
-6.5	0.0	19.4	27.1	0.0	33.1	21.4	19.0	16.6	26.1	20.4
-6.0	11.9	53.7	36.4	40.7	55.3	50.3	26.6	43.1	43.5	42.1
-5.5	38.5	74.6	55.1	62.8	70.4	72.5	53.1	62.6	58.0	58.2
-5.0	70.6	89.6	73.7	83.2	82.1	87.2	70.0	75.3	73.2	76.6
-4.5	78.3	95.5	83.9	91.2	88.4	94.3	85.3	90.2	85.4	87.2
-4.0	88.1	97.0	92.4	97.3	94.3	98.3	93.0	96.9	93.3	94.7
-3.5	96.5	100.0	95.8	100.0	97.4	99.2	97.8	99.2	97.1	98.6
-3.0	100.0	98.5	98.3	100.0	99.5	99.8	100.0	100.0	99.9	100.0
-2.5	100.0	97.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

First extractions

L branches

Ach conc	81.1.e	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h
	0.0	0.0	0.0	0.0	1.2	0.0
	0.0	0.0	0.0	1.6	1.8	0.0
	4.8	4.6	17.6	3.3	2.4	0.0
	6.3	6.8	12.9	5.3	4.2	5.9
	10.1	9.9	17.6	13.1	7.9	7.2
-9.5	10.1	9.9	17.6	13.1	7.9	7.2
-9	10.1	6.1	17.6	13.1	7.9	7.2
-8.5	10.1	6.1	17.6	13.1	7.9	7.2
-8	10.1	6.1	17.6	13.1	7.9	7.2
-7.5	10.1	6.1	17.6	13.1	7.9	7.2
-7	13.2	9.9	21.2	13.1	13.3	11.8
-6.5	15.3	17.9	27.1	24.6	15.2	16.0
-6	25.4	40.3	40.0	46.3	18.2	24.1
-5.5	41.3	57.8	55.3	59.0	34.5	34.6
-5	63.0	75.3	84.7	75.8	60.6	50.6
-4.5	77.2	88.2	103.5	84.4	70.3	68.4
-4	88.4	93.9	117.6	91.0	87.9	81.9
-3.5	93.7	96.6	125.9	95.1	89.7	88.6
-3	97.9	97.3	127.1	95.9	94.5	92.8
-2.5	97.8	97.3	127.1	96.7	94.5	97.0

K branches

81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e
0.0	0.0	38.0	0.0	0.0	0.0
7.0	0.0	22.3	0.0	0.0	0.0
82.9	35.3	52.5	0.0	0.0	16.7
75.9	36.7	41.3	24.7	3.4	14.2
77.8	44.0	62.6	26.0	11.9	21.8
82.9	44.0	62.6	26.0	11.9	21.8
77.8	44.0	62.6	20.0	11.9	21.8
77.8	44.0	62.6	20.0	11.9	21.8
77.8	44.0	62.6	20.0	11.9	21.8
77.8	44.0	62.6	20.0	11.9	21.8
77.8	47.3	67.6	26.7	11.9	24.3
77.8	56.0	68.2	33.3	23.9	36.0
77.8	76.7	69.3	65.3	26.7	52.3
88.0	88.0	72.6	78.7	28.4	69.5
96.8	96.7	86.0	89.3	60.8	84.5
100.0	101.3	88.3	94.7	72.7	93.7
102.5	103.3	94.4	98.7	83.0	100.4
104.4	104.0	96.1	100.0	90.3	103.8
104.4	102.7	98.3	100.0	92.6	104.2
104.4	101.3	92.7	100.0	93.2	103.8

First extractions - continued

K tuber

81.2.h	81.4.h	82.1.e	82.3.e	83.1.b	83.3.b
0	0.0	0.0	1.9	0.0	7.4
0	0.0	0.0	4.8	0.0	6.2
71.0145	58.9	25.0	8.6	20.1	6.6
71.0145	61.3	33.7	11.0	14.5	9.9
65.94203	59.7	31.7	21.4	20.7	13.2
71.0145	61.3	33.7	21.4	20.7	13.2
63.76811	59.7	31.7	21.4	20.7	13.2
63.76811	59.7	31.7	21.4	20.7	13.2
63.76811	59.7	31.7	21.4	20.7	13.2
63.76811	59.7	31.7	21.4	20.7	13.2
63.76811	59.7	31.7	23.8	20.7	15.7
63.76811	62.1	31.7	29.5	27.9	20.7
67.3913	63.7	35.6	53.3	33.5	42.6
74.63768	79.0	46.2	62.9	41.3	59.5
81.88406	91.9	50.5	81.0	76.0	78.5
89.85507	96.8	61.5	91.4	86.0	90.9
94.2029	100.8	90.4	100.0	93.9	96.7
96.3768	101.6	97.1	105.2	100.6	100.8
97.82609	101.6	100.0	107.1	103.4	102.5
97.82609	101.6	97.1	107.1	103.9	102.5

M branches

81.1.b	81.3.b	82.1.h	82.3.h	83.2.e	83.4.e
0.0	0.0	0.0	0.0	77.1	0.0
0.0	0.0	0.0	2.9	86.3	0.0
22.2	0.0	0.0	2.9	79.7	43.5
16.2	0.0	0.0	2.9	77.1	23.9
16.2	2.3	11.7	5.9	79.7	40.2
22.2	2.3	11.7	5.9	86.3	43.5
15.0	0.0	0.0	5.9	79.7	40.2
15.0	0.0	0.0	5.9	79.7	40.2
15.0	0.0	0.0	5.9	79.7	40.2
15.0	0.0	0.0	5.9	79.7	40.2
15.0	3.8	0.0	14.6	79.7	40.2
19.8	13.1	17.6	18.5	79.7	47.3
19.8	34.6	30.2	44.9	79.7	64.1
24.0	52.3	43.9	62.9	83.0	79.3
50.3	76.2	68.3	78.0	91.5	87.5
71.3	87.7	79.0	88.3	94.1	92.4
92.8	96.2	90.2	97.6	96.7	95.7
106.0	100.0	96.6	101.5	98.7	97.3
112.0	100.0	100.0	103.4	99.3	97.3
113.2	100.0	100.0	103.4	99.4	97.3

Second extractions - 1 year later

L branches

Ach conc	133.1.e	134.1.e	135.2.k	135.4.k	136.1.h	136.3.h
	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	25.2	7.5	0.0	0.0
	0.0	20.7	49.0	77.6	48.5	30.1
-9.5	0.0	20.7	49.0	77.6	48.5	30.1
-9	0.0	20.7	49.0	77.6	48.5	30.1
-8.5	0.0	20.7	49.0	77.6	48.5	30.1
-8	0.0	20.7	49.0	77.6	48.5	30.1
-7.5	0.0	20.7	49.0	77.6	48.5	30.1
-7	1.3	20.7	49.0	77.6	48.5	30.1
-6.5	5.3	20.7	53.1	77.6	48.5	30.1
-6	9.3	27.6	65.0	80.6	56.4	50.4
-5.5	20.0	42.5	74.1	86.6	67.8	63.7
-5	37.3	62.1	86.7	92.5	81.9	76.1
-4.5	56.0	75.9	95.1	100.0	90.7	83.2
-4	76.0	89.7	100.0	103.0	96.0	87.6
-3.5	89.3	94.3	102.8	103.0	98.7	89.4
-3	93.3	97.7	104.9	104.5	99.6	89.4
-2.5	97.3	100.0	104.9	104.5	100.0	87.6

K branches

133.2.e	134.4.e	135.1.e	136.2.h	136.4.h
0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	45.0	0.0
0.0	0.0	6.3	58.6	0.0
6.3	6.1	14.6	82.9	39.7
6.3	6.1	14.6	82.9	39.7
6.3	6.1	14.6	82.9	39.7
6.3	6.1	14.6	82.9	39.7
6.3	6.1	14.6	82.9	39.7
6.3	6.1	14.6	82.9	39.7
6.3	16.0	25.5	82.9	39.7
6.3	24.4	42.7	82.9	41.1
15.1	34.4	67.7	82.9	50.7
31.7	58.8	83.3	82.9	68.5
59.5	75.6	94.8	82.9	82.2
73.8	89.3	102.1	86.5	90.4
84.9	100.0	110.4	93.7	95.9
91.3	104.6	112.5	95.5	98.6
92.9	105.3	113.5	100.0	100.0
93.0	105.0	116.7	100.0	100.0

Second extractions - continued

K tubers

133.2.e	134.4.e	135.1.e	136.2.h	136.4.h
0	0.0	0.0	0.0	0.0
0	0.0	0.0	0.0	0.0
0	0.0	0.0	45.0	0.0
0	0.0	6.3	58.6	0.0
6.349206	6.1	14.6	82.9	39.7
6.349206	6.1	14.6	82.9	39.7
6.349206	6.1	14.6	82.9	39.7
6.349206	6.1	14.6	82.9	39.7
6.349206	6.1	14.6	82.9	39.7
6.349206	6.1	14.6	82.9	39.7
6.349206	16.0	25.5	82.9	39.7
6.349206	24.4	42.7	82.9	41.1
15.07936	34.4	67.7	82.9	50.7
31.74603	58.8	83.3	82.9	68.5
59.52381	75.6	94.8	82.9	82.2
73.80951	89.3	102.1	86.5	90.4
84.92063	100.0	110.4	93.7	95.9
91.26983	104.6	112.5	95.5	98.6
92.85713	105.3	113.5	100.0	100.0
93	105.0	116.7	100.0	100.0

M branches

116.1.b	117.2.b	118.2.d	118.4.d	119.4.f	127.1.d
0.0	0.0	0.0	0.0	0.0	0.0
2.5	0.0	0.0	0.0	0.0	0.0
5.9	0.0	0.0	0.0	29.8	0.0
10.1	3.4	0.6	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
14.3	14.5	1.8	0.0	34.5	0.0
21.0	20.7	10.3	0.0	38.7	4.1
25.2	34.5	13.3	5.0	50.0	5.8
41.2	56.6	23.0	6.3	58.3	13.2
58.0	75.2	28.5	6.3	63.1	24.0
76.5	85.5	33.3	33.8	78.6	41.3
86.6	97.9	43.0	40.0	89.9	65.3
94.1	102.1	57.0	60.0	96.4	99.2
97.5	102.0	69.1	75.0	98.8	114.9
100.0	102.0	86.1	87.5	100.0	121.5
100.0	102.0	95.2	93.8	100.0	124.0

MTT assay - Graham's cells

All growing

	Ltuber	Ltuber	Ltuber	Ktuber	Ktuber	Ktuber	T50	T50	T50	Nst	Nst	Nst	Kbr	Kbr	Kbr
-2	110.7	74.7	87.8	71.2	61.9	74.3	73.2	90.8	89.8				96.2	55.6	56.4
-3	123.9	106.4	90.3	115.1	87.4	96.8	102.6	98.3	107.7	104.6	103.0	125.9	99.7	109.6	109.4
-4	79.8	52.4	46.5	116.6	93.6	100.6	95.5	81.8	75.0	92.0	100.9	91.4	102.5	112.2	101.2
-5	119.2	102.4	101.9	113.8	88.8	106.6	87.9	99.4	106.0	85.5	90.0	91.5	114.1	97.0	99.8
-6	117.0	105.6	108.2	110.7	93.1	98.9	96.2	93.8	107.0	86.2	91.4	90.7	88.9	99.8	86.0
-7	110.7	101.7	94.4	106.8	95.2	94.7	103.6	91.7	96.4	89.8	91.0	100.8	90.2	87.9	105.1
-10	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0			

	Mbranche	Mbranche	Mbranche	Kleaves	Kleaves	Kleaves	M leaves	M leaves	M leaves	Rhoicissu	Measurem	0.0	Rhoicissu	0.0	0.0
-2	64.2	55.3	62.5	63.1	51.2	41.3	41.1	39.4	35.9	248.1			108.1	158.3	90.8
-3	93.6	105.9	96.4	120.2	107.4	108.7	104.5	98.0	109.6	168.5	161.1	277.3	145.7	134.3	87.3
-4	92.3	92.2	93.8	119.0	98.5	101.3	110.6	97.4	96.8	123.7	106.0	105.3	118.6	105.2	91.1
-5	83.9	95.3	86.6	113.2	93.8	86.0	106.3	98.3	109.0	158.6	166.0	119.6	115.9	116.6	97.4
-6	94.2	94.9	88.4	112.3	99.5	90.0	103.7	94.0	95.6	151.3	139.9	94.4	107.0	90.8	83.0
-7	93.5	99.8	88.1	105.5	90.1	90.7	102.9	89.1	91.3	177.2	121.5	113.3	119.7	84.3	80.2

	Rhoicissu	0.0	0.0	Rhoicissu	0.0	0.0	Rhoicissu	0.0	0.0
-2	86.7	64.8	60.9	108.9	159.5	170.8	67.4	83.0	92.4
-3	94.9	63.2	47.8	114.9	91.1	89.9	88.7	89.1	86.8
-4	112.7	78.2	83.5	100.3	121.0	125.6	98.0	84.1	138.0
-5	78.3	69.3	87.7	91.5	78.1	126.9	84.8	65.8	88.2
-6	72.3	85.8	93.4	84.0	109.3	102.3	77.1	105.6	89.7
-7	105.6	94.5	103.9	80.3	78.9	99.9	97.5	89.3	76.7

All dormant

	Ctuber	Ctuber	Ctuber	Ptuber	Ptuber	Ptuber	Qtuber	Qtuber	Qtuber	Gr	Gr	Gr	Croots	Croots	Croots
-2	83.7	70.7	46.0	92.5	76.3	69.2	66.9	59.0	78.1	67.3	50.3	63.8	61.4	50.5	80.2
-3	113.2	99.3	112.3	83.0	75.2	132.6		109.2	109.4	101.1	73.7	71.4	70.9	78.2	98.3
-4	129.2	106.7	100.0	112.8	105.1	113.4	83.2	91.4	114.8	111.2	104.7	89.0	105.3	109.7	110.0
-5	103.6	126.7	93.0	92.1	88.9	99.4	84.9	96.6	102.6	113.2	106.2	75.3	75.5	99.8	102.0
-6	121.1	81.0	92.1	90.0	72.3	96.4	87.2	99.3	113.9	109.7	102.7	100.9	72.8	84.7	100.4
-7	114.8	80.2	97.9	90.1	90.1	105.5	78.4	98.7	102.5			58.6	91.0	102.5	95.9

	Pr	Pr	Pr	Qroots	Qroots	Qroots	Pst	Pst	Pst	0.0
-2	150.1	71.5	77.7	67.7	76.0	91.3	42.9	72.1	51.3	95.4
-3	139.2	92.0	71.6	70.8	85.5	110.0	79.1	102.5	107.8	96.0
-4	209.1	164.0	113.1	101.6	122.9	131.5	104.5	118.5	113.0	92.3
-5	191.3	154.0	112.8	125.6	120.6	128.2	92.4	100.9	108.2	102.0
-6	196.2	161.3	111.3	103.9	102.8	122.5	99.2	81.4	101.9	90.0
-7	163.1	143.0	104.3	92.4	89.5	112.8	89.7	98.6	101.6	103.9

MTT assay - Leydig cells

All growing

	Groots	Groots	Groots	T50	T50	T50	Kleaves	Kleaves	Kleaves	Mleaf	Mleaf	Mleaf	Ltuber	Ltuber	Ltuber
-2	132.9	135.1	115.8	61.7	110.6	101.7	109.1	110.5	104.4	131.4	151.9	130.0	141.2	126.0	129.5
-3	174.7	147.6	119.5	96.7	172.8	99.2	209.1	150.0	97.4	129.2	148.1	155.9	133.5	151.0	97.3
-4	120.0	130.9	102.7	60.7	130.3	68.3	190.3	91.5	75.7	170.4	199.0	174.5	105.5	91.7	97.6
-5	109.4	129.8	96.6	63.7	104.3	94.0	128.5	121.0	85.1	100.0	213.8	115.9	119.9	115.1	95.4
-6	130.6	166.0	58.6	109.8	111.0	105.7	100.0	96.0	84.2	167.3	119.0	143.2	44.1	84.9	80.5
-7	97.6	122.5	88.0	61.2	128.7	144.9	86.0	139.1	62.6	111.1	95.2	118.2	156.3	105.8	95.7

	Ktuber	Ktuber	Ktuber	Mbr	Mbr	Mbr	Kbr	Kbr	Kbr	Nst	Nst	Nst	Rholcissus K stems		
-2	160.3	135.3	193.8	244.8	252.1	76.9	153.6	141.7	151.8	130.2	251.1		135.5	126.8	152.7
-3	191.9	152.4	137.8	166.7	117.4	95.5	97.7	156.7	138.2	157.0	288.7	106.3	129.6	142.7	128.8
-4	118.4	84.8	170.5	157.0	105.4	69.7	166.8	102.1	89.5	139.6	205.0	99.6	114.3	123.3	108.9
-5	194.1	116.5	209.8	203.6	267.1	147.2	189.5	181.8	182.5	131.9	176.6	112.6	100.6	131.3	124.4
-6	135.3	131.1	123.8	155.2	239.5	108.6	173.2	158.3	113.2	146.0	183.0	183.4	98.9	114.8	96.7
-7	132.4	75.4	135.2	124.8	107.8	126.9	137.7	132.1	160.5	188.5	256.3	155.3	104.6	132.6	114.9

	Rholcissus K tubers									Rholcissus N stems					
-2	100.7	151.6	111.1	53.0	138.9	44.7	113.4	93.2	101.4	102.8	97.3	98.1	249.0	93.0	97.5
-3	81.4	140.3	121.4	95.3	98.0	100.8	100.0	98.4	131.4	62.7	48.6	57.4	135.3	102.7	101.0
-4	60.9	89.4	95.6	87.4	88.9	94.5	100.8	98.3	100.3	66.5	67.7	55.1	192.4	104.6	79.5
-5	64.6	126.9	103.7	79.0	85.3	94.2	95.3	105.9	84.0	78.4	25.5	74.5	173.0	92.9	91.5
-6	64.2	98.4	111.6	86.0	88.1	104.2	126.5	84.8	93.1	65.8	62.6	79.6	159.8	98.3	121.0
-7	67.9	96.2	92.8	90.0	78.4	87.5	91.1	88.7	88.5	78.5	81.6	90.4	116.7	81.1	77.7

All dormant

	Qr	Qr	Qr	Proots	Proots	Proots	Croots	Croots	Croots	Ptuber	Ptuber	Ptuber	Qtuber	Qtuber	Qtuber
-2	97.1	138.2	100.2	175.9	202.3	129.7	235.1	233.0	123.7	122.4	232.9	189.6	147.3	107.8	169.3
-3	100.3	270.0	130.8	164.7	226.9	127.4	130.8	178.7	97.1	119.8	240.2	161.0	198.4	58.0	83.3
-4	76.3		116.9	158.2	163.2	100.8	208.7	224.8	104.9	106.1	185.8	180.9	153.8	43.9	79.9
-5	98.0	112.0	87.0	101.8	131.0	108.1	116.3	131.7	63.3	109.9	174.0	191.2		128.3	77.4
-6	58.5	197.9	103.0	95.9	176.0	100.8	77.4	129.6	72.6	72.7	199.1	151.8	128.3	87.6	46.4
-7	83.6	111.6	80.0	110.6	138.6		84.1	103.5	47.8	72.1	143.4	110.8	122.8	55.8	67.5

	Ctuber	Ctuber	Ctuber	Pst	Pst	Pst	Rholcissus C tubers						Rholcissus P stems		
-2	88.3	125.1	419.7	169.1	117.7	79.5	81.3	72.4	141.7	87.0	112.9	101.7	126.7	121.6	157.4
-3	81.6	166.4	276.2	170.9	163.6	154.5	190.2	123.1	81.9	108.7	99.9	116.7	111.1	112.5	146.6
-4	106.8	175.7	131.1	93.2	81.0	88.9	101.3	120.6	75.3	86.9	87.5	108.2	87.8	71.8	143.4
-5	143.2	217.4	242.0	144.7	160.8	120.2	118.8	106.2	76.8	88.1	81.2	87.4	85.9	88.3	109.3
-6	111.2	117.0	103.6	162.3	162.0	91.5	137.1	131.0	126.5	140.3	81.8	98.3	100.5	98.2	143.8
-7	137.4	116.2	107.3	129.6	83.9	81.2	145.8	114.9	69.1	101.2	99.6	104.2	120.2	80.6	110.0

Rhoicissus P tubers

-2	182.2	165.6	175.1	110.3	105.3	122.7	115.9	155.8	200.9
-3	171.1	113.1	126.5	76.1	94.7	117.2	116.7	118.9	112.1
-4	120.2	80.2	92.0	109.6	86.1	101.1	89.5	109.1	75.1
-5	120.9	93.8	87.8	69.7	67.6	80.5	95.3	128.1	23.7
-6	123.4	97.7	100.0	93.4	89.4	105.9	85.5	123.6	29.6
-7	151.6	91.7	97.1	63.3	71.3	85.0	76.5	106.1	88.0

MTT assay - Hepatocytes

All growing

	Rhoicissus N stems					Rhoicissus K stems						Rhoicissus M stems			
-2	177.9	201.2	198.3	144.6	243.5	103.6	110.9	163.2	147.6	132.8	165.3	111.3	114.6	112.7	117.2
-3	104.3	106.0	108.2	110.0	121.7	82.1	112.3	125.3	131.2	117.4	123.7	111.7	107.9	102.3	108.4
-4	97.4	115.0	112.1	112.1	156.8	83.6	85.9	130.9	106.7	106.2	96.4	111.0	100.7	105.1	102.1
-5	88.8	104.4	97.9	102.0	159.1	88.6	85.9	109.5	110.1	117.1	92.9	90.3	101.6	96.7	99.3
-6	89.9	104.7	103.7	103.7	179.3	86.0	78.8	108.6	91.7	104.7	96.4	89.8	95.6	98.9	96.4
-7	78.7	108.1	105.7	92.7	124.5	79.6	89.4	112.1	90.9	93.1	93.5	102.7	100.1	98.7	97.5

	Rhoicissus K tubers								
-2	107.5	122.6	143.2	48.2	67.1	56.5	73.4	89.7	137.0
-3	105.5	98.5	138.4	88.9	100.4	100.2	92.7	92.4	113.9
-4	102.4	126.2	110.2	83.2	64.3	94.7	87.1	101.2	96.8
-5	98.7	117.6	125.7	89.0	96.2	93.9	92.7	103.2	98.1
-6	98.1	101.0	125.0	92.2	89.4	94.3	87.0	96.5	104.3
-7	97.2	91.9	107.0	91.4	92.5	99.0	98.6	98.2	92.3

All dormant

	Rhoicissus C tubers					Rhoicissus P tuber						Rhoicissus P stems			
-2	129.1	144.9	151.1	79.0	145.0	113.2	117.6	95.2	93.8	168.2	113.4	110.0	88.3	115.4	127.5
-3	160.3	143.2	150.5	63.9	130.6	107.9	123.2	101.3	89.4	133.4	104.9	105.4	114.3	123.5	135.4
-4	132.2	132.9	145.1	60.0	117.8	96.6	86.0	91.2	82.1	99.0	64.8	91.2	80.5	93.5	119.8
-5	121.6	122.0	133.9	123.9	111.1	99.8	109.8	93.0	86.7	86.1	102.8	93.8	70.5	102.1	114.1
-6	115.8	113.6	129.3	57.2	106.3	94.1	98.6	96.1	84.3	112.3	79.4	98.6	73.0	112.4	119.7
-7	119.6	118.9	129.4	110.3	106.1	96.4	86.0	99.2	90.7	88.7	110.0	96.8	60.7	98.8	95.8

	Rhoicissus Q tubers								
-2	135.3	130.5	84.2	166.7	135.3	113.2	108.4	172.7	126.5
-3	137.2	141.0	96.8	141.8	151.6	102.6	111.4	135.6	102.8
-4	126.8	141.0	88.8	121.7	109.9	85.7	91.8	121.6	83.7
-5	104.2	144.6	65.8	119.6	103.4	75.8	92.4	128.7	94.9
-6	141.9	134.2	79.6	126.5	114.0	92.2	94.0	112.6	99.7
-7	90.4	97.7	78.7	108.2	88.4	91.7	92.5	113.3	93.2

MTT assay - Histiocytoma celis

All growing

540	Ctuber	Croots	Gr	Ctuber	Croots	Gr
-2	187.9	311.8	117.8	130.5	270.5	96.9
-3	103.2	120.0	106.2	123.5	195.6	109.6
-4	97.8	87.2	93.2	110.6	109.5	83.0
-5	106.7	99.2	100.4	108.8	89.6	81.6
-6	97.9	101.2	103.0	116.1	88.1	83.6
-7	91.0	99.8	102.6	121.0	88.0	86.1

All dormant

540	Ptuber	Qtuber	Qroots	Pr	Pst	Ptuber	Qtuber	Qroots	Pr	Pst
-2	172.7	140.8	130.2	159.2	95.2	163.2	105.3	98.7	152.2	73.8
-3	124.4	104.5	102.2	129.1	104.6	177.2	96.4	117.0	142.8	88.0
-4	102.1	97.4	66.3	76.8	98.1	114.6	80.2	84.3	95.2	82.3
-5	96.3	96.5	99.1	100.3	100.5	87.3	89.9	87.5	84.7	90.4
-6	86.5	67.9	104.5	99.8	98.8	88.2	89.3	87.3	82.7	72.6
-7	86.5	101.2	105.6	110.3	100.5	97.1	87.9	92.9	87.7	89.1

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SHORT COMMUNICATION

The Pharmacological Action of *Rhoicissus tridentata* on Isolated Rat Uterus and Ileum

L. C. Katsoulis,* D. J. H. Veale and I. Havlik

[1]Department of Experimental and Clinical Pharmacology, University of the Witwatersrand Medical School, Parktown, South Africa

Decoctions and infusions of *Rhoicissus tridentata* subsp *cuneifolia* (Vitaceae) roots and lignotubers are widely used in South African traditional herbal remedies during pregnancy. The pharmacological action of an aqueous extract of *R. tridentata* subsp *cuneifolia* roots was investigated using isolated rat uterus and ileum. The extract directly stimulated concentration dependent contractions of the uterus and ileum. Pretreatment with the muscarinic antagonist atropine (40 nM) and the cyclooxygenase inhibitor indomethacin (5 μM) both blocked the contractile response to the *Rhoicissus* extract suggesting that muscarinic receptors and cyclooxygenase metabolites could be involved in the contractile response to the extract. The serotonergic antagonist methysergide (1 μM) and the α-adrenoceptor antagonist prazosin (2 μM) had no effect on the direct action of the extract. This infers that the contractile response to the plant extract is independent of serotonin receptors and α-adrenoceptors. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Rhoicissus tridentata; Vitaceae; childbirth; traditional remedies; muscarinic receptors; prostaglandin synthesis.

INTRODUCTION

Rhoicissus tridentata (L.f.) Wild & Drum. subsp *cuneifolia* (Eckl & Zeyh). N. R. Urton, is a deciduous shrubby creeper in the family Vitaceae (Urton *et al.*, 1986), commonly known as wild grape or isiNwazi (Zulu). The leaves are trifoliate with wedge-shaped leaflets. Decoctions and infusions of *R. tridentata* roots or lignotubers are taken orally or as enemas in many South African pregnancy related traditional herbal remedies (Varga and Veale, 1997; Watt and Breyer-Brandwijk, 1962; Veale *et al.*, 1992; van Wyk *et al.*, 1997).

The use of traditional remedies during pregnancy has been associated with premature delivery and growth retarded babies (Morris and Mdlalose, 1991), unexplained fetal death resulting from premature labour (Broster, 1981), increased incidence of meconium stained amniotic fluid, increased incidence of caesarean section and decreased Apgar scores (Mitri *et al.*, 1987). Most of the above birth complications are caused, or contributed to, by increased uterine contractions. Therefore, the pharmacological action of *R. tridentata* was investigated by measuring contractions of isolated rat uterine muscle.

The aim of this investigation was not to look for potential therapeutic agents, but rather to investigate whether the use of *R. tridentata* to induce labour by traditional healers is pharmacologically justified and whether *R. tridentata* has the potential of causing the birth complications listed above.

* Correspondence to: Dr L. C. Katsoulis, Department of Experimental and Clinical Pharmacology, University of the Witwatersrand Medical School, Parktown, South Africa.
E-mail: 054LCKAT@chiron.wits.ac.za

MATERIALS AND METHODS

Plant extract preparation. *R. tridentata* subsp *cuneifolia* roots were collected from Treasure Beach Grasslands, Kwazulu-Natal in July and October 1995 (voucher specimen number — 086931, C. E. Moss Herbarium). The roots were dried, milled and boiled in distilled water for approximately 1 h to mimic the traditional healer's preparation methods. The extract was settled overnight at 4 °C. The supernatant was siphoned off, frozen and lyophilized. The yield of lyophilate was 4.6% (w/w) relative to the dried milled material.

Isolated organ preparations. Virgin Sprague-Dawley rats weighing about 250 g were oestrogenized with stilboestrol (Maybaker (SA)) i.p. 24 h before being killed with CO_2. Portions of uterus and ileum (1.5 cm) were mounted in 50 mL organ baths containing Tyrode solution. The baths were aerated with 5% CO_2 in O_2. The ileum was maintained at 37 °C, whereas the uterine tissue was maintained at 26 °C to decrease spontaneous contractility (Kumagai *et al.*, 1952). Isotonic contractions of the organs were measured and recorded electronically using labographs. The organs were challenged with cumulative doses of reference agonists or herbal extract, or pretreated for 5 min with either an antagonist, the herbal extract, or both before cumulatively adding the

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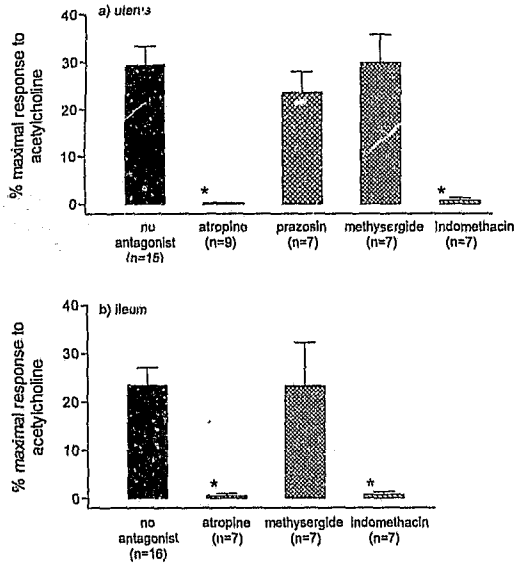


Figure 1. The maximal response of isolated rat (a) uterine and (b) ileal muscle to 1.3×10^{-3} g/mL *R. tridentata* extract (solid bar) compared with the organ response to the plant extract after the organ was pretreated with the above antagonists for 5 min. * $p < 0.05$ significantly different from the plant extract alone.

reference drug. The maximal responses to the plant extract were calculated as a percentage of the maximal response to acetylcholine. The bar graphs were drawn using GraphPad Prism©. Student's *t*-tests were done to test for significant differences. $p < 0.01$ was considered significant.

Drugs used. Reference drugs used in the study were acetylcholine chloride (Sigma), atropine sulphate (BDH), oxytocin (Syntocinon, Sandoz), propanolol (Sigma), noradrenaline bitartrate (Sigma), indomethacin (Sigma), serotonin creatinine sulphate (Sigma), methysergide maleate (RBI) and prazosin (RBI). All stock solutions were made up in Milli-Q double distilled water.

RESULTS

The *Rhoicissus* extract stimulated concentration dependent contractions of both isolated rat uterus and ileum (data not shown). Pretreatment of both uterine and ileal tissue with the muscarinic antagonist atropine (40 nM), and the cyclooxygenase inhibitor indomethacin (5 μM) blocked the contractile response of the organs to the plant extract. The serotonergic antagonist methysergide (1 μM) had no effect on the uterine or ileal response. Prazosin (2 μM), an α-adrenergic antagonist, after the β-adrenergic blocker propanolol (2.7 μM), had no effect on the uterine response to the plant extract (Fig. 1). Preincubation of uterine tissue with the plant extract had no effect on the response to cumulative additions of oxytocin (1 nM–0.3 mM) (data not shown).

DISCUSSION

The results provide pharmacological evidence justifying the ethnic use of *R. tridentata* subsp *cuneifolia* for the initiation and augmentation of labour and that the potential exists for the plant decoctions to cause birth complications caused by increased uterine contractility.

Blocking of the action of the plant extract by atropine and indomethacin

The contractile response to the plant extract of both ileum and uterus was blocked by pretreatment of the organs with the muscarinic antagonist atropine. This infers that muscarinic receptors are involved in the contractile response to the extract. The direct action of the plant was also inhibited by pretreatment with the cyclooxygenase inhibitor indomethacin, suggesting that cyclooxygenase metabolites, such as prostaglandins or thromboxane A_2, are involved in the contractile response to the plant extract. The involvement of muscarinic receptors and the production of cyclooxygenase metabolites were also found to mediate smooth muscle contractility in response to venom from the soldierfish (*Gymnapistes marmoratus*) (Hopkins *et al.*, 1997). Prostaglandin $F_{2\alpha}$, prostaglandin E_2, and muscarinic agonists enhance Ca^{2+}-induced contraction of myometrium in late gestation (Izumi *et al.*, 1996) through G protein stimulation of phospholipase C (Hardman *et al.*, 1996). These contractile elements produce additional contractile force providing expelling forces for delivery of fetuses (Izumi *et al.*, 1996).

Involvement of serotonergic, adrenergic and oxytocic receptor systems

Methysergide had a negligible effect on the direct action of the plant in the uterus or ileum suggesting that serotonergic receptors do not play a role in the mediation of the uterine or ileal response to the *Rhoicissus* extract. Similarly, prazosin did not alter the uterine response, indicating the α-adrenoceptors are also not involved in the uterine contractions induced by the extract. No conclusions can be drawn on the involvement of the oxytocic receptors as an oxytocin antagonist was not used.

CONCLUSIONS

This study provides evidence to suggest that *R. tridentata* subsp *cuneifolia* aqueous root extracts are able to stimulate contractions of isolated rat uterus and ileum directly, and that the contractile response of the uterus and ileum to the extract is mediated predominantly by the muscarinic receptor system and the production of cyclooxygenase metabolites.

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Short Communications

Rhoicissus tridentata subsp. *cuneifolia*: the effect of geographical distribution and plant storage on rat uterine contractile activity

L.C. Katsoulis

Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, Johannesburg, Republic of South Africa
E-mail: 054LCKAT@chiron.wits.ac.za

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Introduction

This study investigated the geographical variation in the contractile activity of crude water decoctions of *Rhoicissus tridentata* (L.f.) Wild & Drum. subsp. *cuneifolia* (Eckl & Zeyh). N.R. Urton, as used by traditional healers. The extracts were tested on isolated rat uterus. The results indicate that the uterotonic activity varies considerably depending on where the plant material was harvested. Most plant extracts stimulated varying degrees of contraction where the extract of one plant inhibited acetylcholine induced contractions. The effect of storing dried material at room temperature and away from sunlight was also investigated. Storing dried plant material does not alter the activity of crude water extracts.

Keywords: *Rhoicissus tridentata*, Vitaceae, spatial variation, distribution, storage, herbal remedy.

Rhoicissus tridentata (L.f.) Wild & Orum. subsp. *cuneifolia* (Eckl & Zeyh). N.R. Urton, is a deciduous shrubby creeper in the family Vitacea, commonly known as wild grape (Smith 1966) or isiNwazi (Zulu) (Gerstner 1941). *Rhoicissus tridentata* is a polymorphic species which has undergone numerous nomenclature revisions over the last two centuries. Five different species, *R. cuneifolia, R. erythrodes, R. cirrhiflora, R. pauciflora* and *R. dimidiata* have been grouped together as *R. tridentata*, but the species has been divided into two subspecies, being subspecies *tridentata* and subspecies *cuneifolia* depending on the number of indentations on the leaflet margins (Urton *et al.* 1986).

Roots and tubers of *Rhoicissus tridentata* are widely used by Zulu traditional healers for stomach ailments, kidney and bladder complaints, epilepsy, infertility, dysmennorrhoea, induction of labour and the facilitation of childbirth (Watt & Breyer-Brandwijk 1962; Bryant 1966; Pujol 1990; Hutchings *et al.* 1996). The use of the plant in pregnancy is the focus of this investigation which is the reason contractile activity on uterine tissue was measured.

Even though considerable work is being done to encourage traditional healers to grow their own medicinal plants (Mender *et al.* 1998), this is not yet the custom. Urbanisation has led to the generation of the industry of plant gathering. Formerly plant harvesting was done by the traditional healers themselves, however, urbanisation has increased the commercialisation of the herb trade. Now plant collecting is often done by untrained commercial gatherers who harvest plants from all over southern Africa and supply plants to Herbal Markets in urban areas (Cunningham 1988). Not only is this system having a disastrous effect on the populations of many popular plant species (Cunningham 1988), but seldom do healers know where or when the plant material was harvested (Pers comm).

The nature and quality of secondary metabolites can be affected by temperature, rainfall, aspect, length of day, light quality and altitude (Evans 1996). Therefore, this study was done to investigate whether there was any variation in the contractile activity of plants harvested from different localities, and whether storage altered the activity of the plant extracts. Both these possible areas of variation could impact on the effect manifested in the patients.

Lignotubers are the part used by traditional healers, but are very difficult to locate as they are interspersed along the roots which usually grow within crevices between rocks. Stem extracts were shown to have the uterotonic activity most similar to that of lignotuber extracts, following the same seasonal variation as lignotuber extracts (Katsoulis *et al.* 1999a). The contractile activity of root extracts was shown to be less than that of extracts from lignotubers and stems. Stems and roots were used for this investigation into the distributional variation in uterotonic activity of extracts from *R. tridentata*.

Methods

Plant collection - distributional variation

R. tridentata subsp. *cuneifolia* stems were collected during summer, which is the season during which the plants yield the most active aqueous extracts (Katsoulis *et al.* 1999a). The plant material was harvested from around the south eastern part of South Africa, from a variety of different habitats (Table 1). The identity of all specimens was verified by L. Katsoulis. Voucher specimens were lodged in the C.E. Moss Herbarium. (Specimen numbers given in Table 1).

Table 1 Collection details of plants harvested to determine the effect of distribution on the contractile activity of aqueous extracts

Location (Voucher specimen number)	Province	Vegetation type[a]	Season	Part	Altitude (m)[b]	MAR[c] (mm)[b]
Suikerbosrand Nature Reserve (086929)	Gauteng	Mixed grassveld	Autumn	stems	1541	698
Mondeor (J090060)	Gauteng	Sour grassveld	Autumn	stems	1541	698
Magaliesburg Mountains	Gauteng	Bushveld gorge	Summer	stems	1157	659
Umlazi (086931)	Kwazulu-Natal	Coast-belt scrub forest	Summer	roots	8	1022
Durban	Kwazulu-Natal	Coast-belt scrub forest	Summer	roots	8	1022
Morgenzon Bos	Mpumalanga	Climax forest in sourveld gorge	Autumn	stems	1525	905.8

[a]Vegetation classification according to Acocks (1988) [b]Supplied by the South African Weather Bureau
[c]MAR = mean annual rainfall

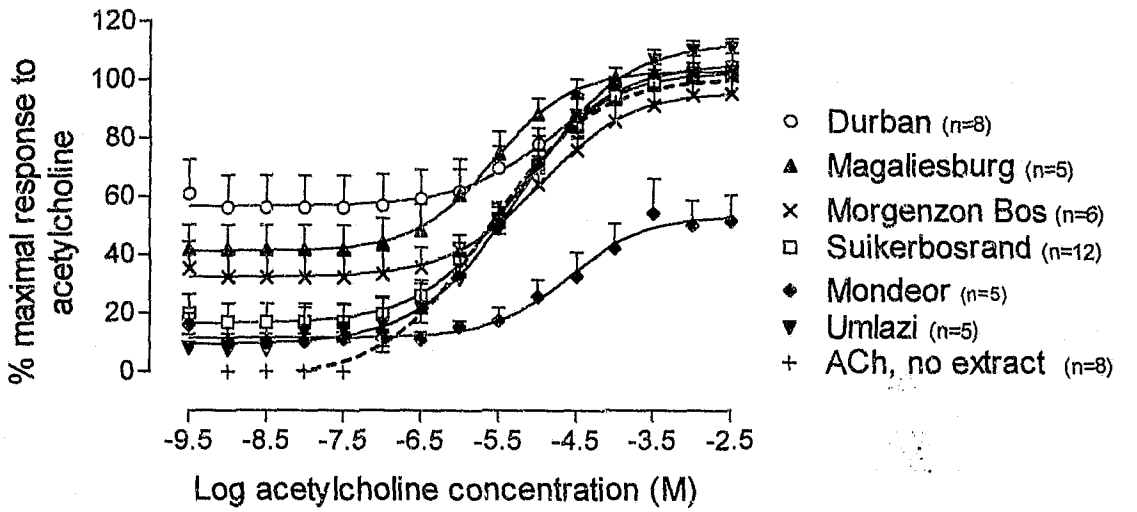


Figure 1 The contractile response of isolated uterine smooth muscle to aqueous extracts of *R. tridentata* plants harvested from different localities around South Africa. The organs were incubated with 1.3 mg/ml crude plant extract for 5 minutes before the cumulative addition of acetylcholine, except for the control curve (+), where acetycholine (ACh) only was added.

Plant collection - effect of storage

Stems and tubers of *R. tridentata* subsp. *cuneifolia* were collected from Suikerbosrand Nature Reserve as for Katsoulis *et al* (1999a). Dried milled samples were extracted one month after being harvested. The milled material was then stored away from direct light, in closed but not airtight containers, at room temperature (25–28°C) for a further eleven months before the second extractions were done.

Milled samples were boiled for an hour to mimic the methods used by traditional healers (± 100 g milled sample per litre double-distilled milli-Q water). The decoctions were settled overnight at 4°C. The supernatant was lyophilised then reconstituted at fixed concentrations in water.

Rat uterine tissue was obtained from oestrogenised female Spraque-Dawley rats (approximate weight 250 g), and mounted in Tyrode (physiological solution) at 26°C to decrease spontaneous contractions (Kumagai *et al.* 1952). The organ baths were oxygenated with carbogen (5% CO_2 in O_2). Acetylcholine chloride (Sigma), a cholinergic agonist that stimulates contractions of smooth muscle was added cumulatively as the reference drug. The effect of the plant extracts was tested by incubating the uterine tissue with 1.3 mg ml^{-1} crude aqueous extract for 5 minutes before the cumulative addition of acetylcholine.

Results

Distributional variation

The contractile activity of the aqueous extracts of *R. tridentata* harvested from different localities around South Africa varied greatly (Figure 1). Plants from all localities yielded extracts which had negligible effect on the maximal response of the organs to acetylcholine, except the extract from the plant harvested in Mondeor that halved the maximal response to acetylcholine. The initial response, which indicates the contractile response to the aqueous extract alone also varied. The extract harvested in Durban stimulated the largest contractions, where the samples from Umlazi and Mondeor yielded the least response. The extract from plant material stored for a month yielded a response identical to that from material stored for a year (Figure 2).

Discussion

The results show there is a variation in the pharmacological response to aqueous extracts from *R. tridentata* plants harvested from different localities. All the extracts augmented the initial response of the organs to low dose acetylcholine, but the extent of augmentation varied. The effect on the maximal response is where the interesting difference occurred. The extract from the plant harvested in Mondeor (Figure 1) was the only extract to shift the dose response curve to the right and inhibit the response to maximal concentrations of acetylcholine, which suggests the extract is competing with acetylcholine for binding sites on cholinergic receptors. Most of the extracts from *R. tridentata* appeared to act as agonists (activate receptors) where the extract from Mondeor had results similar to those expected from an antagonist (block the effect of an agonist). This means different specimens of the same subspecies displayed opposite mechanisms of action. The contractions appear to be mediated through muscarinic receptors (Katsoulis *et al.* 1999b) which are present on most involuntary muscle, including the heart, lungs and intestines. In relation to the above results, one may postulate that extracts from one plant may cause contraction of smooth muscle ,causing diarrhoea, where extracts from a plant from another region may cause smooth muscle relaxation leading to constipation. However, this would have to be confirmed *in vivo*.

The Mondeor extract seemed to compete with acetylcholine in a non-competitive manner. This inhibition of acetylcholine was negated by rinsing the bath inferring that the inhibition is reversible.

Extracts from *R. tridentata* roots were shown to have lower contractile activity than extracts from *R. tridentata* stems (Katsoulis *et al.* 1999b). The root extract from the plant harvested from Umlazi gave a response similar to that of extracts from stems harvested from Suikerbosrand (Figure 1). However, the roots harvested from Durban yielded an extract that stimulated contractions significantly higher than the contractions stimulated by the Suikerbosrand stem extracts.

Even though these results have illustrated the variation with distribution, no conclusions can be made about what factors may be responsible for this variation. Soil samples, the mean rainfall, temperature and the rate of photosynthesis prior to harvesting

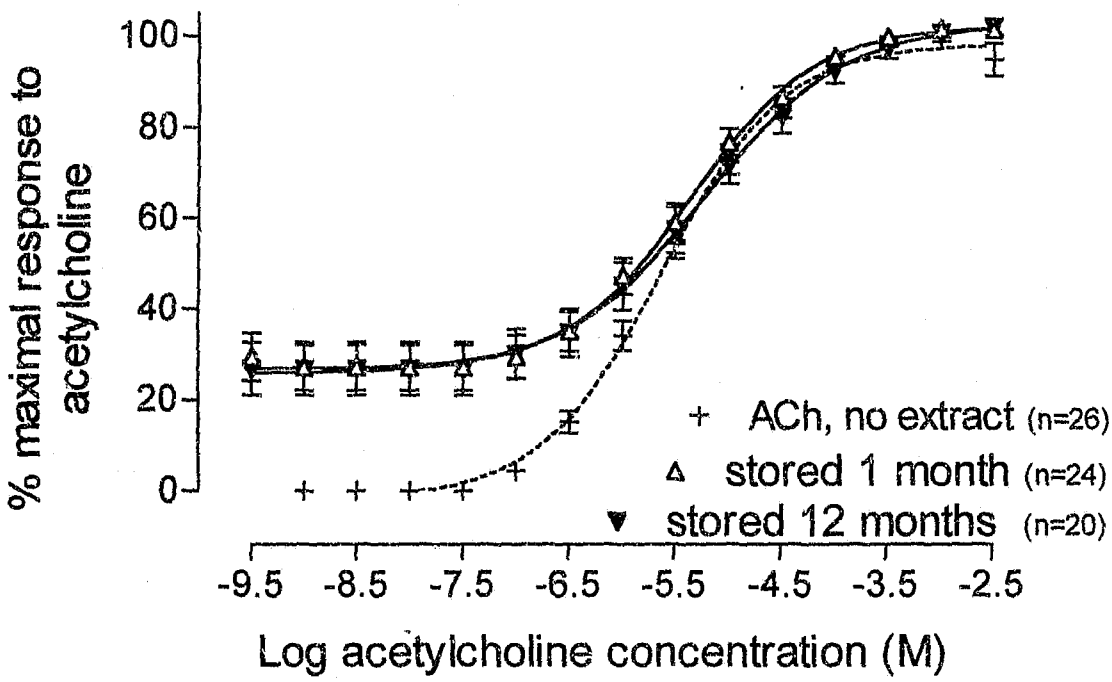


Figure 2 Uterine contractile response to *R. tridentata* extracts from plant material that had been stored for 1 month () compared to extracts from plant material that had been stored for 12 months (). The organs were incubated with 1.3 mg/ml of plant extract for 5 minutes before the cumulative addition of acetylcholine, except for the control curve (+) where only acetylcholine (ACh) was added. Each point represents the mean of the response of organs from 20 to 24 rats with the SEM.

were not obtained. Further studies using controlled environments, such as in a phytotron, would be necessary to ascertain which factors influence the composition of compounds within the plant that are extracted in water.

The specimens tested did not exhibit much variation in gross morphology. The leaf shapes and sizes were very similar. The only difference in morphology was that the foliage of the specimen from Mondeor, which had antagonistic activity, was a lot more dense. Chemotaxonomic studies should be done to investigate whether the species delineation proposed by Urton (1986) is valid chemically. Perhaps the variation in these results is a manifestation of taxon differences which are not manifested in leaf morphology.

These results support the suggestion by Tobler (1994) that there should be a holistic approach to standardising the production of medicinal plants. The entire production process of medicinal plants should be controlled, starting from the cultivation and continuing the control through the processing of the plant material. Collecting the plants from 'the wild' predisposes the patient to risks of toxicity through variation in the degree and type of response to the plant remedy. This distributional variation is compounded by the seasonal variation shown in Katsoulis *et al.* (1999a).

The results on the effect of storing dried plant material indicate that the active components of the plant that are extracted by water are stable at room temperature, and away from sunlight (Figure 2). This is relevant to the traditional healer as dried plant material is stored by medicinal plant vendors and by the traditional healers themselves. The results suggest that storing plant material does not alter the efficacy of the remedy.

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The status of *Larryleachia* Plowes and additional synonymy for *Lavrania* Plowes

P.V. Bruyns
Bolus Herbarium, University of Cape Town, 7701 Republic of South Afrcia

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The genus *Larryleachia* Plowes is shown to be superfluous and is reduced to synonymy under *Lavrania* Sect. *Cactoidea*. Full synonymy for the four species placed by Plowes in *Larryleachia* is given. *Lavrania* sect. *Cactoidea* is validated. A neotype is selected for *Lavrania marlothii* (N.E.Br.) Bruyns.

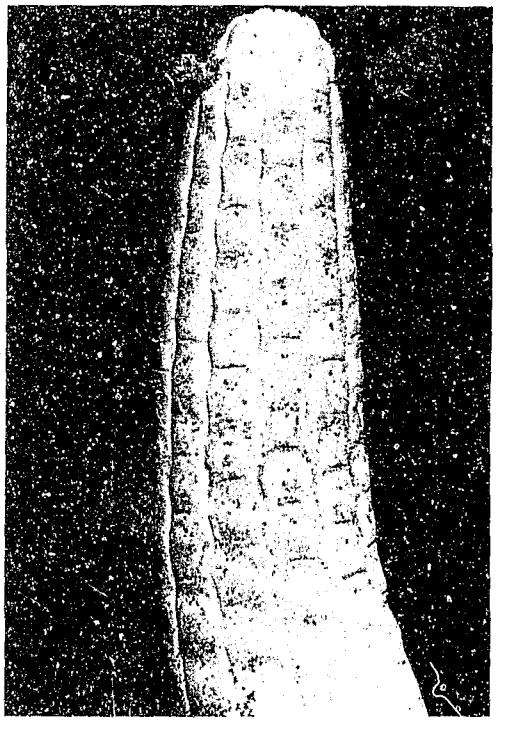
It is necessary to examine the 'revision' of Plowes (1996), where he proposed that the genus *Lavrania* (*sensu* Bruyns 1993) be split into two genera and the four species of *Lavrania* Sect. *Cactoidea* moved into a genus of their own (*Larryleachia*) with *Lavrania* once more monotypic (i.e. *sensu* Plowes 1986). It is particularly worrisome that this has been taken up uncritically in the recently published 'List of southern African Succulents' of the National Botanical Institute (Smith *et al.* 1997). As the generic arrangements within the stapeliads are a cause for concern at present (see also Walker 1997), it is felt that the case of *Larryleachia* should be dealt with properly. I have, therefore, again examined the available material and revisited the arguments on which my concept of *Lavrania* was based and, if anything, this exercise has convinced me that my original treatment was correct and that *Larryleachia* is unnecessary.

I wish to examine the arguments put forward by Plowes (1996), where 12 'differences' between *Lavrania* and *Larryleachia* were enumerated. Each of them will be considered in detail (throughout, the page numbers refer to Bruyns (1993) and 'L.' refers to *Lavrania*).

1. 'In *Larryleachia* the flowers are borne in small groups of 1–3 that open successively...usually on young growth near the apex of the primary as well as other stems. In *Lavrania* the inflorescence is a fascicle of 6–15 flowers [which]...are not borne at the apex of the stems but low down near the base of new stems. Furthermore the primary stem does not bear flowers.'

In *L. perlata* (p. 261) the flowers may be in groups of up to 12 per inflorescence. In the other three species, they arise in groups of 1–5 (6) per inflorescence (p. 246, 258). The number of flowers is, therefore, not diagnostic. In addition, in *L. perlata* several flowers may be open at once on an inflorescence and the term 'fascicle' may be applied here too.

In the second place the position of the inflorescences is not as





Figures 1 and 2 *Lavrania haagnerae* [Namibia, *PVB 4069* (BOL)]. Two different stems, each with an inflorescence near its apex. There are other inflorescences near the base of the stems.

This paper has been accepted for publication in the South African Journal of Medicine.
(Recommended changes to the paper have not been included).

Seasonal variation in uterotonic activity of *Rhoicissus tridentata* extracts

L.C. KATSOULIS *, D.J.H. VEALE AND I. HAVLIK
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, Johannesburg, South Africa.
* Corresponding author; e-mail: 054LCKAT@chiron.wits.ac.za

ABSTRACT

Rhoicissus tridentata lignotubers are widely used in Southern African traditional pregnancy related remedies.

Objectives: To determine the seasonal variation in contractile activity of extracts from different parts of the plant.

Methods: Isolated rat uterus tissue was used to compare the contractile activity of crude aqueous extracts of *R.tridentata* made from plant material harvested every three months for two years.

Results and Conclusions: The activity of the plant extracts from plants harvested in summer and autumn are 4-5 fold higher than extracts from plants harvested in winter or spring. The tubers stimulate the largest contractions, followed by the stems, roots then the leaves. These results highlight the need to standardise the timing of harvesting *R.tridentata*.

Keywords: *Rhoicissus tridentata*, Vitaceae, seasonal variation, pregnancy related remedies, herbal remedies, plant parts.

INTRODUCTION

Decoctions of *Rhoicissus tridentata* subsp *cuneifolia* roots and lignotubers are used by South African women in ethnic herbal remedies for delayed childbirth and to facilitate childbirth[1,2]. *R.tridentata* decoctions are prepared by boiling dried milled roots or lignotubers for approximately an hour by the traditional healer who sells the bottled decoctions (personal communication). The plant is widely distributed throughout the eastern portion of Southern Africa [3], and is found in most traditional healer vending stores around Johannesburg.

Pharmacological investigations using isolated rat uterus and ileum suggest that the contractile response to hot water plant extracts may be mediated by muscarinic receptors and the stimulation of prostaglandin synthesis [4]. While doing preliminary work on the pharmacology of the plant extracts it became evident that there was a high degree of variability in the activity of extracts from different plant samples. *R. tridentata* has been shown to exhibit geographical variation in the pharmacological response to aqueous extracts. The plant extracts acted either agonistically or antagonistically on muscarinic receptors depending on where the plant was harvested [5]. Temporal variation is another possible source of variability in chemical constituents and biological activity of plants. This is a well documented phenomenon [6,7]. The aim of this study was to determine whether the contractile activity of *R.tridentata* varies seasonally.

METHODS

R.tridentata subsp. *cuneifolia* plant material was collected from Suikerbosrand Nature Reserve, 60 km south of Johannesburg. Three plants were harvested every three months from May 1996 to April 1998. The identity of the plants was validated by L. Katsoulis and voucher specimens of the three individual plants harvested were lodged in the C.E. Moss Herbarium (Voucher specimen numbers 086928, 082929 and 082930).

Milled plant material was boiled for an hour, settled and the supernatant was lyophilised. Uterine tissue from oestrogenized virgin Sprague-Dawley rats was mounted in organ baths containing aerated Tyrode solution. The activity of the

extracts was tested by incubating the tissues with 1.3 mg/ml of the various plant extracts for five minutes before adding acetylcholine cumulatively. Isotonic contractions were expressed relative to the maximal response to acetylcholine. Details of the methods are given in a previous publication [8].

Results and Discussion

Figure 1 illustrates how the different parts of the plants harvested in winter and spring all yield aqueous extracts with low levels of contractile activity, where the activity of extracts from different parts of plants harvested during summer and autumn varies considerably. During summer the tubers yield the most active aqueous extracts, followed by the stems then roots and leaves. The autumn plants exhibit a similar pattern of variation except the roots are the least active. The autumn and summer tubers are 4 and 5 times more active than the winter and spring extracts. The stems display similar seasonal variability.

All parts of the plant harvested in summer and autumn are able to directly stimulate contractions of isolated uterus. The traditional healers usually use the lignotubers, which yield the most uterotonically active extracts. The activity of the tuber extracts is the highest during the growing season and decreases during the seasons when the plant is dormant.

The root and stem extracts show a similar pattern in seasonal variation in uterotonic activity to that of the tuber extracts. That is, the activity of the extracts starts increasing when plants are harvested after the first rains. The activity increases throughout the growing season (or rainy season) reaching a peak in autumn. The activity of the extracts then falls as the plants lose their leaves and become dormant during winter. The rise in activity as the rains start could be caused by the improved uptake of minerals from the soil following the mineralization flush after the first rains, which usually leads to a sharp rise in the nitrogen content within the plant [9].

The variability in the contractile activity of the extracts could be caused by a) altered concentrations of the active principle(s), b) a change in the quality or

nature of the active principle(s), or c) changes in the production of compounds such as saponins which may alter the extraction of the pharmacologically active component(s).

Relevance to conservation

The stem extracts yield a similar response to the most active part, the lignotubers. This is significant from an environmental perspective, as harvesting stems for the trade in traditional remedies would have a much lower impact on the plant populations of *Rhoicissus tridentata* than harvesting the lignotubers and roots. With the current use of the lignotubers, the plant has to be uprooted for lignotubers to be harvested.

Clinical Relevance

The variability in the uterine response to the decoctions from different seasons highlights a severe flaw in the use of traditional herbal remedies. Collectors and vendors have no way of determining the chemical composition of the plant material harvested. Eight traditional healers in and around Johannesburg were questioned on whether they were aware of any seasonal variation in the efficacy of *R.tridentata*, none of them seemed aware of the concept.

Traditional healers were also unaware of any toxic effects of the plant (personal communication) despite reports implicating the plant in fatal poisonings [10, 11]. This suggests that only certain plant samples may be toxic. The possibility exists that the toxicity of the plant also fluctuates seasonally which would explain why the traditional healers are generally unaware of the toxic potential of *Rhoicissus tridentata.* Further investigation should be done to determine whether the poisonings that have occurred are concentrated at a certain time of year.

The variability in the pharmacological activity of *Rhoicissus* also reiterates the importance of standardisation criteria that need to be established for herbal remedies. The results also support the suggestion made by Tobler [12] that there should be a holistic approach to standardising the production of medicinal plants, by controlling the entire production of medicinal plants. Starting from the cultivation and continuing the control through the processing of the plant material.

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Table 1. Statistical differences between the contractile activity of extracts from *Rhoicissus* from different plant parts harvested in different seasons.

Tubers		**Roots**	
spring and summer	**	spring and summer	**
spring and autumn	***	summer and autumn	**
summer and winter	***	autumn and winter	*
autumn and winter	**	**Stems**	
		spring and summer	***

Key: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

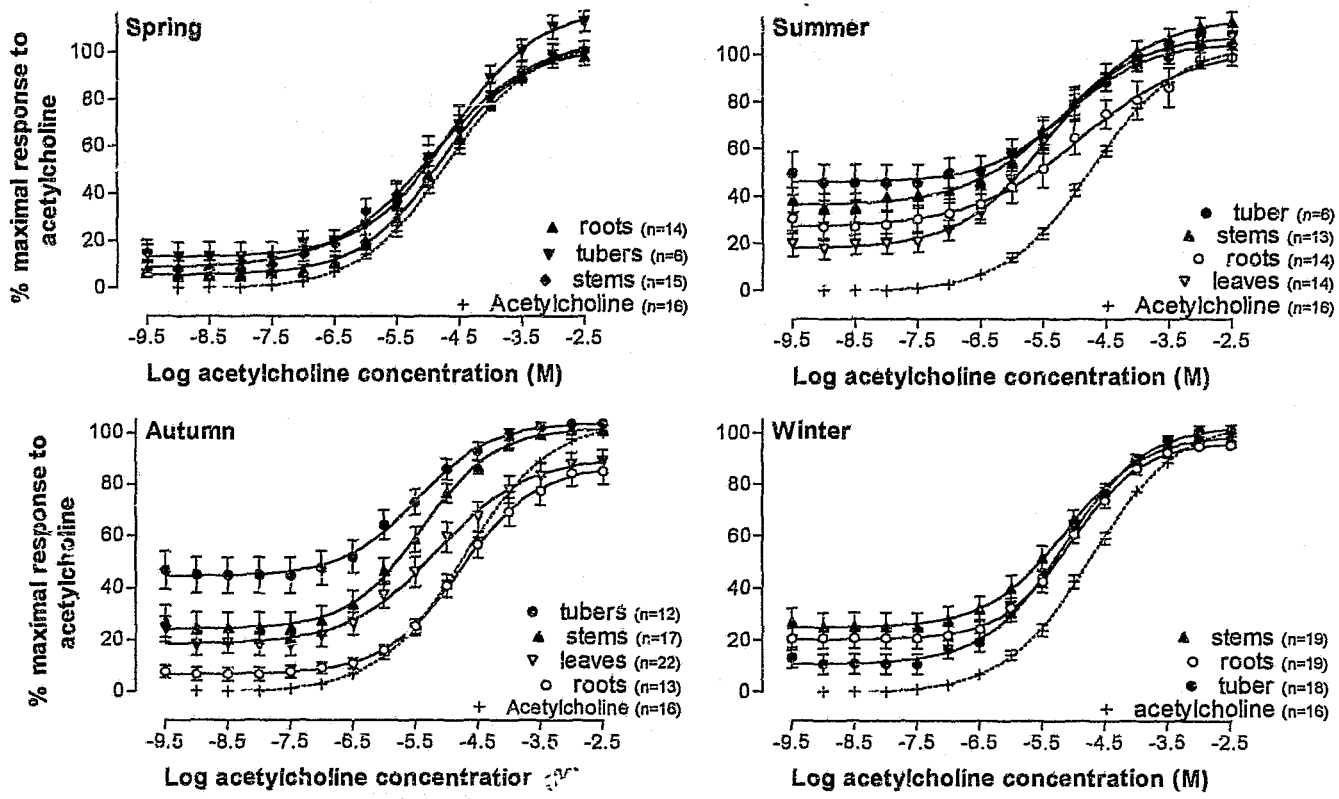


Figure 1. Dose response curves of isolated rat uterus when pre-treated with 1.3 mg/ml aqueous extracts of all parts of *Rhoicissus tridentata* harvested during spring before the cumulative additions of acetylcholine. Each point represents the mean with the vertical bars representing the SEM.

Uteroactive constituents from *Combretum kraussii*

K. Bridget Brookes*[a], Olga V. Doudoukina[a], Lynn C. Katsoulis[b] and D. Joy H. Veale[b]
[a]Technikon Mangosuthu, P.O. Box 12363, Jacobs 4026, Republic of South Africa
[b]Pharmacology Department, University of the Witwatersrand, 7 York Road, Parktown, 2193, Republic of South Africa

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We have established that certain extracts of *Combretum kraussii* Hochst roots cause contractions in isolated rat uterine tissue, and have purified the most active uterotonic components. The mode of action of the various extracts/components on isolated rat uterus falls into three different categories. The activity of crude extracts from leaves and branches of *C.erythrophyllum* (Burch.) Sond. and *C. kraussii* Hochst was also measured. We have identified the following compounds from root extracts: combretastatin, combretastatin A-1 and combretastatin B-1 as well as the corresponding 2-*O*-β-D-glucosides of the latter two combretastatins, β-sitosterol, ellagic acid and the di- and trimethyl ethers of ellagic acid. This is the first report of the occurrence of combretastatin and ellagic acid derivatives in this *Combretum* species.

*Corresponding author

The efficacy of Combretaceae growing in tropical and sub-tropical areas as medicinal plants was established in the 1980's, when the anti-cancer activity of this family was demonstrated by a survey of stilbenes and bibenzyls isolated from heartwood of *Combretum caffrum* utilising the National Cancer Institute's biological evaluation system.[1,2] Combretastatin A-1 was particularly potent. Subsequently, stilbenes, bibenzyls and their glucosides having the ability to inhibit cell growth were isolated from *C. kraussii* seeds.[3] The pharmacological action of such components was demonstrated on dorsal root ganglion neurones, human myotubes and muscle tumour cells. Combretastatin B-1 produced remarkable effects on the action potentials of excitable cells.[4]

C. kraussi roots, or '*umdubu*' in Zulu, are commonly used in traditional medicines in South Africa, where approximately 80% of the Zulu and Xhosa population relies on such medications to treat a variety of illnesses. *C. kraussii* is one of the commonly used plant roots in '*isihlambezo*', a general term for pregnancy-related herbal medicines. Aqueous decoctions of roots from several plants, selected from a total of approximately sixty varieties, are prescribed by traditional healers to promote a favourable course of pregnancy and a quick uncomplicated labour. Such herbal medicines are also common in other countries, for example, China, Japan, Hawaii, India and North America. However, South Africa lags behind other countries in researching traditional medicines. Larsen and coworkers[5] documented the effectiveness of an *isihlambezo* medicine by demonstrating that a solution of the herbal mixture had an oxytocin-like effect on isolated rat uterine tissue, causing electrically measured contractions. Veale *et al.* and Kaido *et al.* found that aqueous extracts of *Clivia miniata*, *Agapanthus africanus*, *Pentanisia prunelloides* and *Gunnera perpensa*, all used in *isihlambezo* decoctions, stimulated smooth muscle contraction in isolated rat uterine tissue.[6-8] We have established that aqueous extracts of *C. kraussii* roots are also uteroactive, and have purified the two most active phenolic components. Full structural elucidation of these components is in progress, so at present we report those compounds characterised in our search for the active components.

Experimental

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Gemini 300MHz spectrophotometer. TMS was used as internal standard for ^{1}H NMR spectra, and the central line of the deuterated chloroform triplet at δ 77.07 for ^{13}C NMR spectra. IR spectra were recorded on a Pye Unicam SP3-200 infrared spectrophotometer on samples dispersed in KBr discs. HPLC data were recorded on a Millipore Chromatograph using a Lichrosorb RP 18 NC-04 column (column I; particle size 5 μm, column dimensions 250 × 4 mm ID); or a Thermo Separation Products (TSP) Spectraseries HPLC using a Grom-sil 100 ODS-O AB column (column II; particle size 5 μm, column dimensions 250mm × 4mm ID) or a Hypersil C18-RP H5ods column (column III; column dimensions 250 × 4 mm ID). Sugar analyses were carried out on a Waters Carbohydrate Analysis HPLC column (column IV; 125 Å 10 μm, dimensions 300 × 3.9 mm). GC/MS spectra were recorded on a Hewlett-Packard 5890 Series II Spectrophotometer with a 5971 Series Mass Selective detector. UV spectra were recorded on a Pye Unicam PU 8800 UV/VIS spectrophotometer. Melting points, uncorrected, were determined on a Kofler hot stage apparatus. Mass spectral determinations were carried out using EI and FAB MS techniques on a VG 70-70E instrument.

Isolated Organ Experiments

Uterine strips were dissected from virgin female Sprague-Dawley rats and were set up in 50 cm^3 isolated organ baths as per the methods of Veale *et al.*[6] and Kaido *et al.*[8]

Plant material, extraction, chromatography and identification of constituents

Combretum kraussii roots were obtained from Pigeon Valley Nature Reserve, Durban, in June 1995 (voucher specimen no. 121751 at the Natal Botanical Gardens Herbarium). All extracts were prepared by boiling plant material with approximately ten times the volume of solvent under reflux for an hour. The extract prepared with hot methanol, comprising 8.57% of material from 3 kg of dried and milled roots of *C. kraussii*, was separated by column chromatography on Merck silica gel 60 (230–400 mesh). TLC was performed on aluminium-backed Merck silica gel 60 plates. Silylation was carried out on 1–2 mg of freeze-dried sample by heating with bis(trimethylsilyl)acetamide (300 µl) in sealed vials at 60–80°C for 1 h. Excess derivatising reagent was removed in a N_2 stream, and the sample was dissolved in $CHCl_3$ for GC/MS procedures.

Small-scale extractions using other solvents and plant parts were also carried out for organ tests on crude extracts. *C. erythrophyllum* (Burch.) Sond. leaves and branches were collected in Magaliesburg, whereas leaves and branches of *C. kraussii* Hochst were collected from the Pretoria National Botanical Gardens. Both species were harvested in March 1996. All plant material was dried away from direct sunlight, milled and boiled in water for an hour to mimic the preparation techniques followed by local traditional healers. The infusion was lyophilised, then reconstituted in constant concentrations. The structures of the isolated components were elucidated using ^1H NMR, ^{13}C NMR, UV, IR, MS, GC/MS and HPLC spectroscopic methods. Unless otherwise specified, the purity of components isolated was greater than 95%. The following components were eluted from a silica gel column in order of increasing solvent polarity:

β-Sitosterol **1**

Elution from a silica gel column with hexane-ether (2:1) produced 1.78 g (0.37%) of **1**, based on the dry plant material. Recrystallisation from methanol afforded colourless plates m.p. 142–144°C, (lit.,[9] m.p. 138–139°C), M^+ 414.713 for $C_{29}H_{50}O$. The monoacetate of **1** was prepared by heating in pyridine-acetic anhydride for 1 h at 100°C. Recrystallisation from methanol gave a pure product, m.p.122–123°C (lit.,[10] 124–125°C), M^+ 456.751 for $C_{31}H_{52}O_2$. Compound **1**, identified as β-sitosterol by comparison of optical rotation and IR, ^1H NMR and ^{13}C NMR spectra with literature data,[9,10] is widely distributed in higher plants.

Combretastatin **2**

A pure specimen of compound **2**, eluted from the column with chloroform/methanol (20:1), was recrystallised from diethyl ether to produce colourless needles, m.p. 135–137°C (lit.,[11] 130–131°C), M^+ 334 for $C_{18}H_{22}O_6$. Compound **2** was identified as combretastatin by comparison of optical rotation, IR, ^1H NMR , ^{13}C NMR and FAB-MS data of **2** with literature data for combretastatin.[11] Pettit *et al.*[11] separated combretastatin from *C. caffrum* roots, but until now there have been no other reports of the presence of combretastatin in other Combretaceae species. Acetylation of **2** with acetic anhydride / pyridine at 90 for 6 h, and recrystallisation from methanol/water, gave colourless needles, m.p. 126–130°C, M^+ 418 for $C_{22}H_{26}O_8$. Combretastatin diacetate has not previously reported.[11]

3,3',4-Tri-O-methylellagic acid **3**

Compound **3**, a white solid eluted from the column with chloroform/methanol (20:1), was washed with methanol and recrystallised from dioxane to yield shiny colourless needles, m.p. 312–315°C (lit.,[2] 303–306°C), M^+ 344 for $C_{17}H_{12}O_8$. UV:λ_{max} (MeOH) 243, 355 (sh), 366 nm; (MeOH + NaOAc) 246, 390, 401 nm. The IR, ^1H NMR and ^{13}C NMR data were identical to those published for 3,3',4-tri-O-methylellagic acid.[12] It is only possible to distinguish between the closely similar isomers of trimethylated ellagic acid by their UV spectra.[13] The intense absorption at 243 nm was not shifted appreciably on addition of NaOAc, thus clearly establishing that the free hydroxy group was in the 4-position, and that **3** was therefore 3,3',4-trimethylellagic acid.

Combretastatin B-1 **4**

The fraction eluted from the column with hexane/ether (1:5) and having t_R 4.39 min on HPLC in acetonitrile:water (60:40) at 1.0 cm^3 min^{-1} using column I was crystallised from ether/hexane to yield colourless needles, m.p. 99–101°C. UV, ^1H NMR, ^{13}C NMR and IR data were identical to those of combretastatin B-1[1] except for a weak carbonyl absorption at 1635 cm-1 in the IR spectrum. This was probably due to partial oxidation of the catechol unit to the quinone form while grinding crystals of **4** with KBr under an IR lamp. Pettit *et al.*[1] did not report this absorption, but ran a film for their IR spectrum, as they isolated combretastatin B-1 as a gum, as did Pelizzoni *et al.*[3] The ^{13}C spectrum of **4** showed no evidence of a carbonyl group, however. Component **4**, identified as combretastatin B-1, has previously been reported in *Combretum caffrum* roots[1] and *C. kraussii* seeds.[3]

Combretastatin A-1 **5**

Compound **5**, eluted from the column with hexane/ether (1:5), was present in mixtures together with **4**. However, it separated from **4** both by TLC (butanone:$CHCl_3$ 1:1,

R_F 0.88 for **5** and 0.76 for **4**) and by HPLC analysis (t_R 4.15 min using the same conditions as for **4**). Compound **5** had the same mobility as an authentic sample of combretastatin A-1 in each case. The trimethylsilyl derivatives of **5** and **4** also separated on GC/MS analysis, the derivative of **5** having a shorter retention time than that of **4**, and showing a significant molecular ion at *m/z* 476 (base peak) consistent with the stable highly conjugated aromatic stilbene structure of combretastatin A-1.

3,3'-Di-O-methylellagic acid **6**

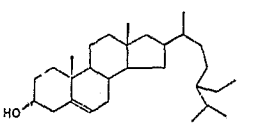
Compound **6** was separated as insoluble solid material from the fraction eluted from the column with chloroform/methanol (10:1); M^- (HRMS) 330.0358 (Calc. for $C_{16}H_{10}O_8$: 330.0376); m.p. *ca.* 280°C (decomp.) (lit.,[12] 338–340°C; lit.,[13] 332–335°C). Compound **6** had t_R 3.30min in acetonitrile : water (60:40) at 1 cm^3 min^{-1} (76.1%); t_R for trimethylellagic acid was 4.31 min under the same conditions. UV: λ_{max} (MeOH) 238, 358 nm; (MeOH + NaOAc) 239, 358, 402 (*sh*) nm. The molecular ion M^- at 330.0358 and the ^{1}H NMR, IR and UV spectroscopic data compared well with data in the literature.[12,14] On the basis of the UV spectrum on addition of NaOAc, we were able to assign the methoxy groups to the 3,3' positions by comparison with literature data.[15]

Ellagic acid lactone **7**

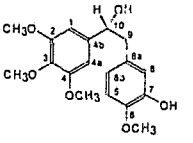
Component **7**, separated from the MeOH/CH_2Cl_2 (1:1) extract (room temperature), was eluted from the column with $CHCl_3$/MeOH (1:1). Recrystallisation from DMF gave khaki-yellow fine needles, m.p. >330°C. UV: λ_{max} (MeOH) 241, 348 nm; (MeOH + NaOH) 239 (sh), 271, 330 (sh), 380 (sh) nm. The identity of **7** as ellagic acid lactone was established by comparison of UV, IR and HPLC data with literature data[15] and an authentic standard.

Combretastatin B-1 glucoside **8** *and combretastatin A-1 glucoside* **9**

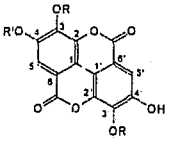
A mixture of these compounds was eluted from the column with chloroform/methanol (10:1) as a dark brown gum in 2.14% yield based on the dry plant material. It was re-chromatographed on silica-gel to yield a chromatographically homogeneous substance (TLC). Hydrolysis of **8** and **9** (5.5 g) was carried out in methanolic HCl (2 mol dm^{-3}) for 6 h under argon. Methanol was then evaporated and the mixture was extracted with ethyl acetate, yielding a solid material (2.2 g) comprising three components. By comparison with standards, these were determined to be combretastatin B-1 **4** (approximately 50%), combretastatin A-1 **5** and a third component, possibly a phenanthrene resulting from ring closure during hydrolysis. Combretastatin B-1 was obtained as colourless crystals from a hydrolysate.



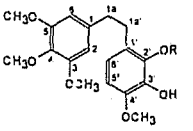
1 β-Sitosterol



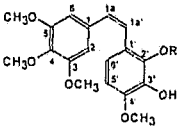
2 Combretastatin



3 R,R'=CH_3 3,3',4-Tri-*O*-methylellagic acid
6 R=CH_3, R'=H 3,3'-Di-*O*-methylellagic acid
7 R, R'=H Ellagic acid



4 R=H Combretastatin B-1
8 R=glucose 2'-*O*-β-D-Glucopyranoside of combretastatin B-1



5 R=H Combretstatin A-1
9 R=glucose 2'-*O*-β-D-Glucopyranoside of combretastatin A-1

HPLC analysis (column IV) of the water layer after hydrolysis showed the presence of glucose [t_R 9.594 min in acetonitrile : water (80:20) at 1.0 cm^3 min^{-1}] by comparison with an authentic glucose standard. By comparison to literature data,[3] the mixture of **8** and **9** was identified as an approximately equal mixture of the 2'-*O*-β-D-glucopyranoside of combretastatin B-1 and the 2'-*O*-β-D-glucopyranoside of combretstatin A-1 respectively by FAB MS and ^{1}H NMR analysis, and also from consideration of the results of acid hydrolysis. Both B-1 and A-1 glycosides were previously identified in *C. kraussii* seeds.[3]

Compounds **10** *and* **11**

These phenolic glycosides, at present not fully characterised, are the most polar substituents and were both obtained as khaki-yellow powders from a hot water extract of roots previously extracted with methanol. Compound **10** was separated first from the concentrated water extract by addition of methanol/acetone (1:1). From the concentrated mother solution after removal of **10**, further addition of methanol/acetone (1:1) precipitated **11**. HPLC in acetonitrile : H_2O (60:40 at 1.0 cm^3 min^{-1}) gave a retention time of 1.59 min for **10** and 2.20 min for **11**.

Results and discussion

A typical HPLC analysis of a *C. kraussii* hot water extract is shown in Figure 1. The crude extracts pre-

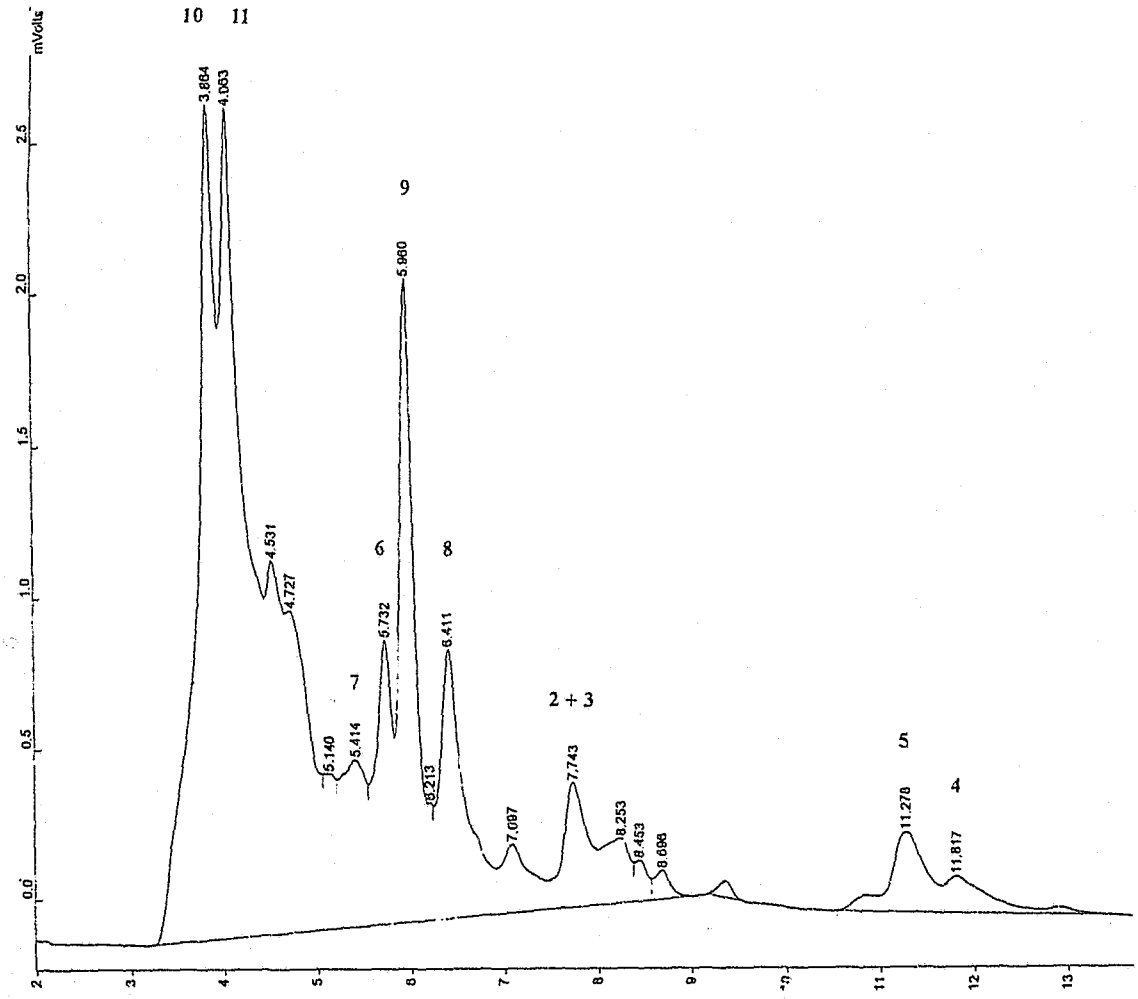


Figure 1 HPLC of aqueous extract of *C. kraussii* roots on column III in acetonitrile : water (60:40) at 0.5 cm^3 min^{-1}. Compound 10, 16.9%; 11, 20.0%; 7, 4.2%; 6, 4.8%; 9, 9.9%; 8, 6.9%; 2 + 3, 3.2%; 5, 2.9%; 4, 1.7%.

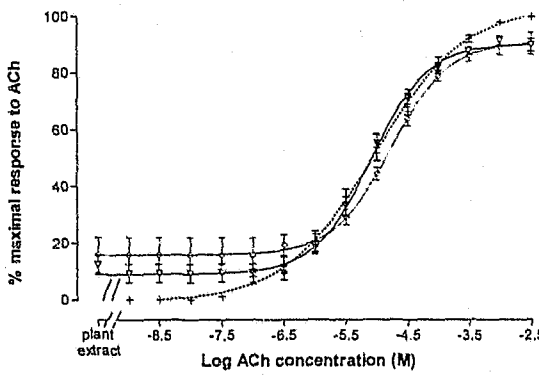


Figure 2 The effect of crude aqueous *C. kraussii* leaf (▽) and branch (◊) extracts on the isolated uterine response to acetylcholine. The organs were treated with the crude extracts 5 min before the cumulative additions of acetylcholine. Each point represents the mean of 4 replicates.

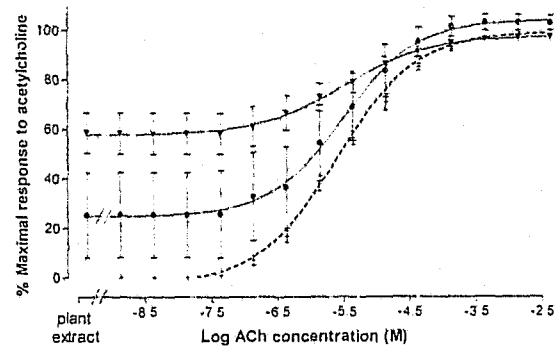


Figure 3 Dose response curves of *C. kraussii* root fractions which augment the response of isolated uterus to acetylcholine without altering the maximal response to acetylcholine. The organs were incubated with 1.3 mg cm^{-3} of compound 11 (□; n = 12) and compound 10 (■; n = 5) 5 min before the cumulative addition of acetylcholine (+; n = 9).

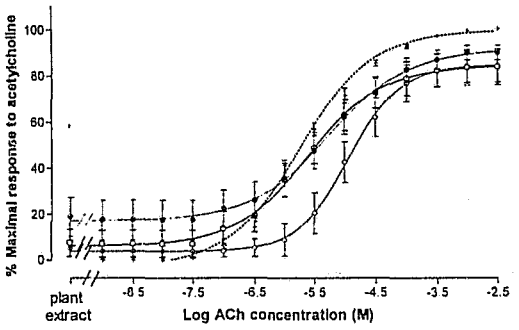


Figure 4 Dose response curves of *C. kraussii* root fractions which augment the response of isolated uterus to acetylcholine and inhibit the maximal response to acetylcholine. The organs were incubated with 1.3 mg cm^{-3} of the following extracts 5 min before the cumulative addition of acetylcholine: compounds 8 and 9 (l; $n = 12$), compound 4 (○; $n = 9$), and compound 1 (◊; $n = 6$). The curves are compared to acetylcholine alone (+; $n = 9$).

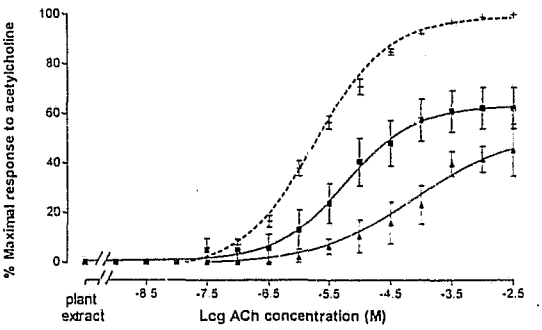


Figure 5 Dose response curves of fractions of *C. kraussii* which do not alter the initial response to acetylcholine but inhibit the maximal response of isolated uterus to acetylcholine. The organs were incubated with 1.3 mg cm^{-3} hot methanol extract (■; $n = 9$) and 1:1 methanol/dichloromethane extract (s; $n = 5$) for 5 min before the cumulative addition of acetylcholine. These curves are compared to acetylcholine alone (+; $n = 9$).

pared in the same way as used by traditional healers stimulated dose-dependent contractions of isolated uterine tissue. Preliminary investigations established that there was no significant difference in the size of the contractions stimulated by either *C. erythrophyllum* or *C. kraussii* extracts. The direct activity of various crude extracts augmented the initial response to cumulative additions of acetylcholine, and the maximal response to acetylcholine was inhibited in all cases as illustrated for *C. kraussii* leaf and branch extracts (Figure 2).

The fractions isolated from *C. kraussii* roots displayed three distinct effects on the response of the isolated uterus to acetylcholine. The first group, comprising compounds **10** and **11**, stimulated contractions of isolated uterine muscle directly. This is illustrated by the first points on the curve representing the response to plant fractions alone (Figure 3). The contractions stimulated by both these compounds were considerably larger than those caused by the crude hot water extracts. Neither fraction had a significant effect on the maximal response to acetylcholine.

The second group, which includes compounds **1**, **4**, **8** and **9**, exhibited a range of direct uterotonic activity and also inhibited the maximal response to acetylcholine (Figure 4). This response indicates that although the initial response is additive, at higher concentrations of acetylcholine the extracts inhibit the organ's response to acetylcholine in a non-competitive manner.

The third group, comprising the hot methanol and methanol/dichloromethane extracts, depressed the maximal response to acetylcholine (Figure 5). These extracts had no direct stimulatory effect on the uterine muscle and suppressed the maximal response of the uterus muscle to acetylcholine. The results imply that these extracts act as non-competitive antagonists and that they could be interfering with the activation of uterine cholinergic receptors in some way.

Preincubation of the organs with compounds **3** and **7** did not alter the uterine response to acetylcholine. In other words, these two compounds neither stimulated contractions directly nor inhibited the maximal response to acetylcholine. The methanol and methanol : dichloromethane extractions make use of solvents not accessible to traditional healers, who use hot water extracts. It is interesting to note that the action of these two extracts differs from those of the hot water extracts, in that they do not directly stimulate uterine contractions and have a marked inhibitory effect on the maximal response to acetylcholine.

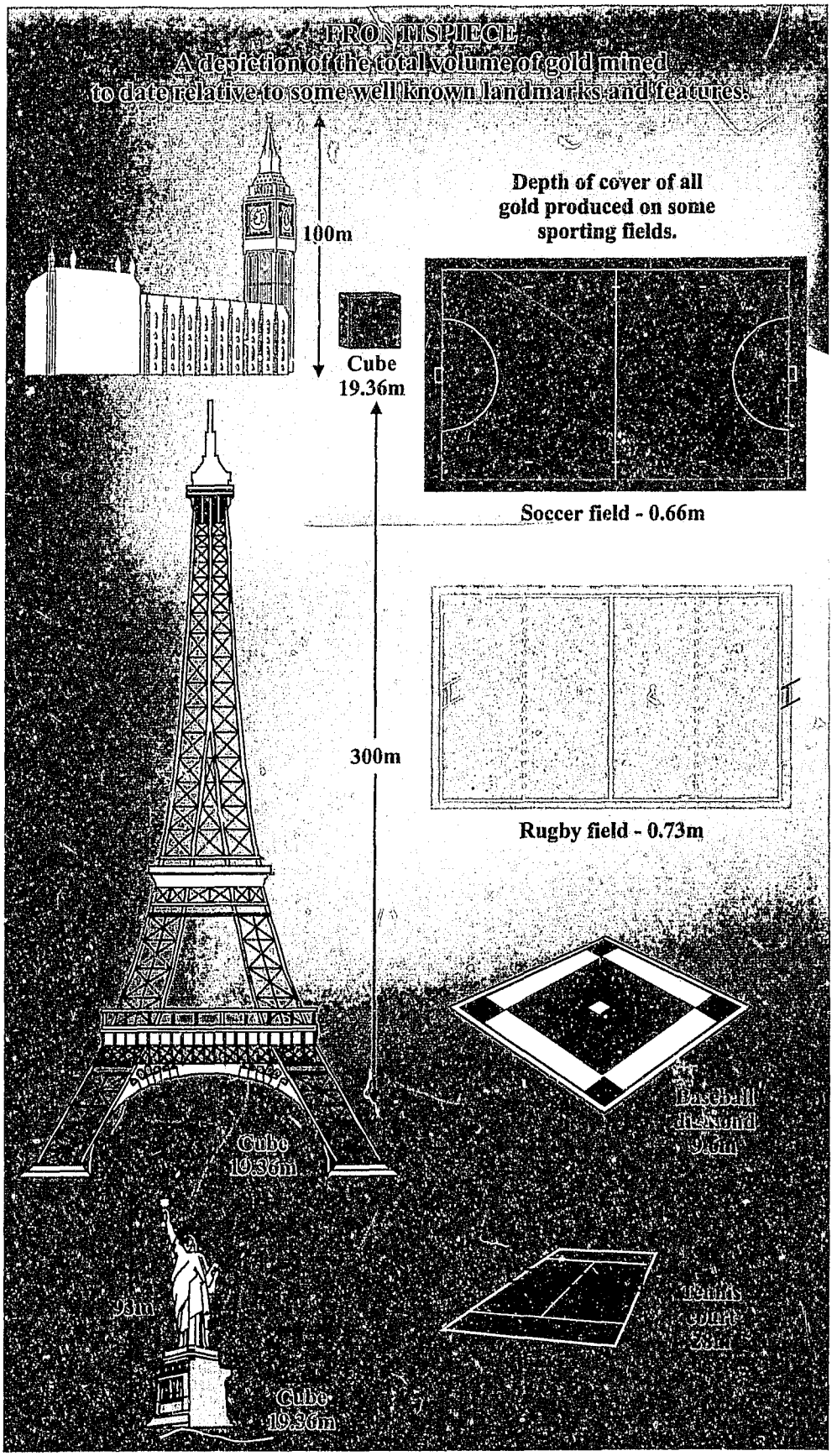
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FRONTISPIECE
A depiction of the total volume of gold mined to date relative to some well known landmarks and features.
100m
Cube
19.36m
Depth of cover of all gold produced on some sporting fields.
Soccer field - 0.66m
Rugby field - 0.73m
300m
Cube
10.36m
Baseball du Nord
9.6m
93m
Cube
19.36m
Tennis court
23m

Author Katsoulis L C
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