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ANTI-FEEDANT EFFECTS OF SURFACE ACCUMULATED FLAVONOIDS OF *POLYGONUM SENEGALENSE*

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ABSTRACT. The leaves, peduncles and petioles of the aquatic weed *Polygonum senegalense* are either coated with resinous material rich in flavonoid aglycones or are covered by quasi crystalline farina but which also contain the same set of aglycones. The crude petroleum ether wash, as well as the chlorofom chromatographic fraction show strong anti-feedant effects against *Schistocerca gregaria*.

INTRODUCTION

Polygonum senegalense Meisn, is a perennial aquatic weed found in some highland regions of East and Central Africa. It may occur as two extreme forms: the glabrous *P. senegalense forma senegalense* or the tomentose *P. senegalense forma albotomentosum*; intermediates between these two forms are also known (1). The aerial parts of *P. senegalense* exudes a yellow substance onto drying paper when pressed. In 1977 it was reported that a hydrophilic extract of the leaves of the plant had significant molluscicidal activity (2). A bioassay directed separation led to isolation of quercetin-3-(2''-galloyglucoside) (1) which at 10 ppm was 100% destructive towards *Lymnae natalensis, Biomphalaria pfeifferi* and *B. glabratus*, snails that are implicated in schistosomal dissemination (3). Maradufu and Ouma also reported the isolation of the non-polar chalcone 2',4'-dihydroxy-3',6'-dimethoxychalcone (2) which at 40 ppm was effective against *B. pfeifferi* and *B. sudanica* (4).

In our quest to chemically survey the eleven Kenyan *Polygonum* species for possible chemotaxonomic correlation of the sort observed for *Rumex* (5) species, we have re-investigated *P. senegalense*. We have found that the leaves, penducles and petioles of the plant are covered with flavonoid aglycones which are biologically active (*vide infra*). These aglycones are found either as a yellow resin or as quasi-crystalline farina leading to the two forms. The occurrence of external flavonoids has been recorded for Asteraceae species especially in xeric habitats (6,7). However this is the first time that an example of this feature amongst the Polygonaceae is reported. Many possible functions have been speculated for these external exudates, including anti-bacterial, anti-fungal, anti-viral and anti-feedant (especially against insects).

In this paper we report the existence of several more non-polar flavonoid aglycones of *P. senegalense* found on its aerial surfaces and provide preliminary results to show that this surface exudate has anti-feedant action against *Schistocerca gregaria*.

RESULTS AND DISCUSSION

The procedure used in extraction was such that only externally deposited material was removed. Fresh leaves or flower heads were dipped in non-polar solvents for upto two minutes; longer periods of exposure usually led to discharge of chlorophyll, an occurrence taken as an indicator for extraction of internally accumulated compounds. Washing with petroleum ether gave a dark gummy extract. The level of the extract varied with age of the leaf with highest concentration being found on young leaves (8-17%). The need for this material seems to diminish once a strong cuticle is developed.

The petroleum ether extract was tested for anti-feedant effect using the procedure of Butterworth and Morgan (8) with sugar soaked filter paper and gave 100% protection against *Schistocerca gregaria* at 10 ppm while the control was virtually all consumed in a 48 hr exposure period (Table 1). A broad band chromatography of the crude petroleum ether concentrate was performed on silica gel using solvents petroleum ether, benzene, chloroform and methanol. Activity was found restricted in the chloroform fraction, F_C (see Table 1).

Table 1. Percentage protection of filter paper impregnated with 0.25 M sucrose solution and dipped in *Polygonum senegalense* leaf wash concentrate or F_C solutions after 48 hrs.

Concentration	% Protection	
mg/ml	Crude extract	F_C
1	100	100
0.1	100	95.6
0.01	100.0	86.5
0.001	98.0	62.2
0.0001	31	34.5
0.0	<2	<2

Table 2. ^{13}C (^1H) NMR chemical shifts (δ, ppm) for chalcones 2-7 and flavanones 8 and 9 at 90.56 (360) MHz.

Chalcone Carbon Numbering	2	3	4	5	7	Flavanone carbon numbering	8	9
1[1]	106.4	104.2	106.3	-	104.4	2	(5.42d)	79.2(5.47d)
2[2]	158.7(14.34s)	158.3(7.38s)	168.4	-	156.5	3	(2.90m)	43.4(3.0m)
3[2]	125.3	135.3	93.8(6.09d)	(5.92d)	94.9(5.92s)	4	-	195.8
4[2]	155.2(6.38s)	150.8	162.5	-	165.6	5	-	168.0
5[1]	89.7(6.06s)	92.7(6.09s)	91.3(5.95d)	(6.00d)	94.9(5.92s)	6	(6.05d)	95.2(6.17d)
6[1]	158.7	163.0(13.91s)	166.2	(14.2s)	156.5	7	-	164.2
7	127.4(7.79d)	143.3(8.12d)	142.3(7.89d)	(7.76d)	30.8(3.0t)	8	(6.07d)	94.3(6.15d)
8	142.5(7.87d)	126.6(7.85d)	127.5(7.77d)	(7.81d)	45.3(3.4t)	9	-	-
9	193.2	192.3	192.6	-	204.4	10	-	-
1	128.4	127.9	135.5	-	141.7(7.30m)	1[1]	-	138.4
2	128.8(7.60m)	128.8(7.63m)	128.8(7.38m)	(7.55m)	128.7(7.30m)	2[1]	(7.27m)	128.9(7.4m)
3	128.4(7.39m)	128.5(7.38m)	128.3(7.38m)	(7.35m)	126.1(7.30m)	3[1]	(7.27m)	126.1(7.4m)
4	130.0(7.39m)	130.2(7.38m)	130.0(7.38m)	(7.35m)	128.8(7.30m)	4[1]	(7.27m)	126.1(7.4m)
5	128.4(7.39m)	128.5(7.38m)	128.3(7.38m)	(7.35m)	126.1(7.30m)	5[1]	(7.27m)	126.1(7.4m)
6	128.8(7.60m)	128.8(7.63m)	128.8(7.58m)	(7.58m)	128.7(7.30m)	6[1]	(7.27m)	128.9(7.4m)

All these fractions were separated further. Several compounds exist in some of them; the total number observed to date (on TLC and isolated) is about twenty. We have so far characterised the eight major ones: the list includes five chalcones, a dihydrochalcone, and two flavononeș. Structural assignments have been made through extensive use of spectroscopic data correlated with literature data (see Table 2 and Experimental). Substitution patterns have been established through NMR nuclear Overhauser experiments and UV shift data. Compound **6** was identified wholly through use of an authentic marker.

In all cases oxygenation of the basic flavonoid skeleton is restricted to A-ring (see Fig. 1). Furthermore the A-ring is either tri- or tetra-oxygenated with each pattern showing different degrees of methylation.

The observed anti-feedant effects that are observed for this easy-to-extract mixture of mostly non-polar chalcones make *P. senegalense* a weed with great economic potential.

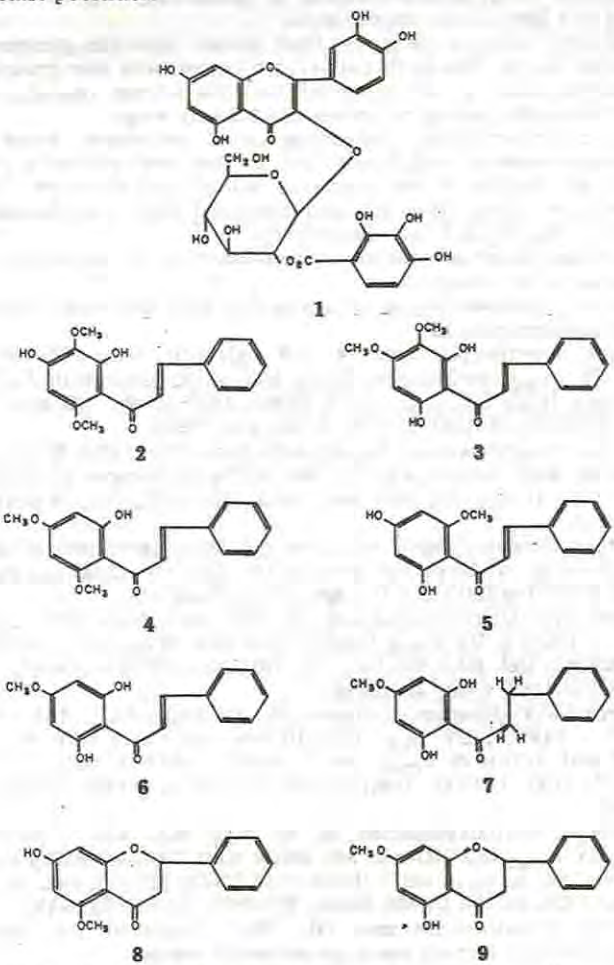

Fig. 1. Flavonoids from *P. senegalense*.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ with TMS as internal standard at 360 and 90.56 MHz, respectively. EIMS direct probe insert was performed at 70 eV. HRMS have been given for M^+ ions for compounds for which they have been determined.

Plant materials. P. senegalense was collected at different times of the year from within Nairobi city (1560 m) and from around L. Naivasha (1895 m). Appropriate samples of *P. senegalense forma senegalense* and *P. senegalense forma albotomentosum* are deposited in the University of Nairobi, Botany Department herbarium. (Voucher No. 89/61 and 89/62, respectively).

Extraction. Freshly collected leaves or flower heads were extracted by dipping them in petroleum ether, dichloromethane or acetone for two minutes. Removal of solvent led to a dark gummy concentrate.

Second and third leaves were picked from several branches, grouped together, and extracted as above. The sixth and seventh leaves were also grouped together and extracted likewise. It was observed that the average deposition on young leaves was 17.3% while that on old leaves was 8.3% by weight.

Isolation and characterisation. Extraction with petroleum ether according to the procedure above of wet leaves (160 g when dry) yielded a dark gummy substance (9.2 g). Elution of this substance (9.0 g) over silica gel (160 g) using solvents petroleum ether (800 ml) and methanol (400 ml) yielded fractions designated as F_P, F_B, F_C and F_M, respectively.

F_P had only one colourless compound which based on the Liebermann-Burchard test was assumed to be a terpenoid.

F_B on further chromatography on silica gel with petroleum ether-benzene mixtures gave two compounds:

2'-hydroxy-4',6'-dimethoxychalcone (**4**, 510 mg). M.p. 89 - 90°C (lit. (9) m.p. 90 - 91°C); UV λ_{max} (MeOH) nm: 230sh, 337, shift latter with NaOMe, $AlCl_3$ (unchanged with HCl); IR ν_{max} cm^{-1} (KBr): 1610 (C=0); MS m/z (rel. int.): 284 (M^+, 31), 207(100), 181(39), 103(34), 95(26) and 77(65).

5-hydroxy-7-methoxyflavanone (**8**, 164 mg). M.p. 110-111°C; UV λ_{max} (MeOH) nm: 284, 320 sh, shift former with NaOMe, $AlCl_3$ (unchanged by HCl); IR ν_{max} cm^{-1} (KBr): 1625 (C=O); MS m/z (rel. int.): 270 (M^+, 55), 193(100), 138(68), 95(96) and 77(52).

F_C on further chromatography on silica gel using petroleum ether, benzene and dichloromethane mixtures and preparative TLC on silica gel, gave several compounds of which the following five have been identified:

2',4'-dihydroxy-3',6'-dimethoxychalcone (**2**, 170 mg). M.P. 104 - 106°C (lit. (4) m.p. 124 - 125°C); UV λ_{max} (MeOH) nm: 342; IR ν_{max} cm^{-1} (KBr): 1618 (C=O); MS m/z (rel. int.): 300 (M^+, 70), 181(100), 195(71), 193(40), 167(66); HRMS: theory 300.1065, found 300.1050.

2',-6'-dihydroxy-3',4'-dimethoxychalcone (**3**, 80 mg). M.P. 145 -146°C (lit. (10) m.p. 147 - 149°C); UV λ_{max} (MeOH) nm: 334 shifts with NaOMe, $AlCl_3$ (not changed with HCL); IR ν_{max} cm^{-1} (KBr): 1615 (C=O); MS m/z (rel. int.): 300 (M^+, 100), 196(78), 168(14) 103(12), 77(10); HRMS: theory 300.1065, found 300.1085.

2',4'-dihydroxy-6'-methoxychalcone (**5**, 40 mg). M.p. 203 - 205°C (lit. (9) m.p. 207°C); UV λ_{max} (MeOH) nm: 341 shifts with NaOMe, $AlCl_3$ (not changed by HCl) and NaOAc; IR ν_{max} cm^{-1} (KBr): 1610 (C=O); MS m/z (rel. int.): 270(M^+, 32), 269(86), 193(42), 95(25); HRMS: theory 270.0877, found 270.0845.

2',6'-dihydroxy-4'-methoxychalcone (**6**). This compound was identified on TLC analysis of extract through use of an authentic marker.

2',6'-dihydroxy-4'-methoxydihydrochalcone (**7**, 55 mg). M.p. 174 - 176°C (lit. (11) m.p. 175 - 177°C); UV λ_{max} (MeOH) nm: 316 sh, 283, the latter

shifts with NaOMe, $AlCl_3$ (not changed with HCl); IR ν_{max} cm^{-1} (KBr): 1625 (C=O); MS m/z (rel. int.): 272(M^+, 25), 193(80), 95(100);HRMS: theory 272.0892, found 272.0845.

F_M on further chromatography on silica gel with mixtures of ethyl acetate and hexane gave compound **9**.

7-hydroxy-5-methoxyflavanone (**9**, 60 mg). M.p. 143 – 144°C (lit. (9) m.p. 144-145°C); UV λ_{max} (MeOH) nm: 284, 316 infl., shifts with NaOMe and NaOAc; IR ν_{max} cm^{-1} (KBr): 1655 (C=O); MS m/z (rel. int.): 270(M^+, 55), 193(19), 166(100), 138(45), 95(12), 77(11).

Antifeedant activity of petroleum ether extract. This was performed according to the procedure of Butterworth and Morgan (8). Discs (5 x 5 cm) of Whatman No. 1 filter paper were soaked in 0.25 M sucrose solution and dried. The test discs were dipped into different concentrations of the test sample dissolved in dichloromethane, while the controls were not treated in this manner. The controls were usually fully consumed by ten 5th instar hoppers in a 48 hr period while the average (2 runs) percent protection by various solutions are shown in Table 1.

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