Bioactive compounds from some Kenyan ethnomedicinal plants: Myrsinaceae, Polygonaceae and *Psiadia punctulata*

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Abstract

There are several described medicinal plants in Kenya from a flora of approximately 10,000 members. Strong cross-medical information from the 42 ethnic groups points to the high potential of some of these species. The Myrsinaceae are well established ethno-anthelmintics and anti-bacterials. They are harbingers of long alkyl side chain benzoquinones which clearly have a protective function from their histochemical disposition. The main benzoquinone in the sub-family Myrsinodae is embelin while for the Maesodae it is maesaquinone together with its 5-acetyl derivative; the distribution of these benzoquinones by their alkyl side chain length or the presence/absence of a 6-methyl group is in accord with morphological sub-family de-limitation. The benzoquinones showed antifeedant, anti-microbial, phytotoxic, acaricidal, insecticidal and nematicidal activity. Many other benzoquinones of medium and minor concentration were also isolated and characterised. Some plants belonging to the Polygonaceae which are widely used as ethno-anthelmintics have been studied. The common anthelmintic anthraquinones were obtained from all five Rumex species while the naphthalenic acetogenin derivative, nepodin was more selectively distributed. The leaf of Polygonum senegalense is up to 17% surface exudate; about thirteen non polar flavonoid derivatives (chalcones, dihydrochalcones, flavanones and a flavone) have been isolated from it. From the internal aerial tissues of this plant, the major flavonoids were common flavonoids, quercetin, kaempferol, luteolin and their glycosides. The only unique compound isolated from this plant was 2'-glucosyl-6'-hydroxy-4'-methoxydihydrochalcone whose aglycone, uvangolatin is part of the exudate mixture. Other leaf exudate plants studied include the stomach-ache medicine, Psiadia punctulata (Compositae) from which novel methylated flavonoids, kaurene and trachyloban diterpenes have been found.

Introduction

More than 1200 Kenyan plant species are recorded as medicinal in Kokwaro's book (1976) out of a floral population of approximately 10,000. Beentje (1994) also alludes to ethno-medical uses of many of these plants, the majority of which have not undergone even initial phytochemical investigation. The WHO has long recognised that traditional medicinal plants could be useful in an integrated health care delivery system of a country. However such plants must not be dangerous, be effective and that preparations are not adulterated or made harmful by parasites and micro-organisms (WHO, 1978).

For medicinal plants to be used alongside modern medicine careful phytochemical, pharmacological and toxicological standardisation for the chosen plants must be instituted so that dosage levels can be described in an informed way. With so much pharmaceutical research going on in drug companies in the Western world, the choice of which disease condition needs to be focused on by third world researchers is a vital question. The focus indeed should be on parasitic diseases rather than problems of physiological disorder such as cancer and viral type that are concentrated on by large pharmaceutical companies of the West. It was in this vein that a phytochemical

Table 1. Traditional uses of Myrsinaceae (Kokwaro, 1976).

Plant	Use
M. africana	2–3 handfuls of fruits are chewed
	for roundworm and tapeworm
	treatment and remedy for chest
	pains and stiff joints. No harm is
	done when too much is taken
E. schimperi	Fruits chewed as both vermifuge
	and purgative. Dried fruits and
	roots are boiled or soaked in water
	and infusion drunk for intestinal
	worms
R. melanophloeos	Fruits used as anthelmintic when
	chewed and eaten in porridge to
	expel intestinal worms
M. lanceolata	Fruits used as purgative to remove
	worms and as remedy for sore
	throats or eaten to cure tapeworm

study of anthelmintic and bacteriocidal plant groups was pursued in our institute as a starting point.

Two of the most important ethno-medical anthelmintic and anti-bacterial families in Kenya with strong cross ethnic group usage are the Myrsinaceae and the Polygonaceae. Forays into studies of members of these groups led to observation of interesting phytochemicals and this spawned studies of other species with corresponding characteristics from different families.

Myrsinaceae

There are five Myrsinaceae species in Kenya – Maesa lanceolata, Myrsine africana, Rapanea melanophloeos, Embelia schimperi and Embelia keniensis. They have been used in ethnopharmacology by many of the country's ethnic groups. Many claims are found about their chemotherapeutic efficacy for human microbiological and protozoan disease management in the traditional settings but the anthelminthiasis reports seemed most widespread (Table 1). It was this anti-intestinal worm effect which served as the initial impetus for our involvement with this family.

A literature search revealed an overflow of reports (e.g., *vide infra*) on the pharmacological efficacy of the extracts of the Myrsinaceae species as anthelmintics and other activities, or the secondary metabolites from them which are 'ubiquitously' benzoquionoid. We have also shown that embelin (4), maesaquinone

(1) lower testosterone levels in white New Zealand male rabbits on intramascular injection (Makawiti and Midiwo, 1991; Githui et al., 1991). Embelin acts as an anti-feedant to *Schistocerca gregaria* and is larvicidal to *Aedes egyptii* larvae (Midiwo et al., 1995).

Previously Watt and Breyer-Brandwijk (1962) referring to old literature claimed the existence of embelin and or rapanone in Maesa lanceolata, Myrsine africana, Embelia keniensis, Embelia schimperi and Rapanea pulchra (This latter species has since been combined with the then R. rododendroides into one species R. melanophloeos; Halliday, 1984). These reports were treated as inadequate and better work was necessary using more recent methods. The major compounds in the more common four species (M. lanceolata, R. melanophloeos, M. africana and E. schimperi) were found to be 2,5-dihydroxydialkyland 2,5-dihydroxyalkylbenzoquinone (Table 2) derivates, maesaquinone (1), acetylmaesaquinone (2), maesanin (3), embelin (4), rapanone (5), (Figure 1) but there are several other minor and medium level compounds. The distribution of the major compounds is such that the first three are restricted to M. lanceolata while the latter three are observed only in the other species (Midiwo et al., 1988). The bias observed is in accord with delimitation of the family into two sub-families - Maesodae (Maesa) and Myrsinodae (Myrsine, Rapanea, Embelia and Ardisia). This observation had already been asserted for Japanese Myrsinaceae (Ogawa and Natori, 1968). The table also shows that these biologically active compounds (there are other reports on their anti-bacterial (Bhatnagar et al., 1961), anti-fungal (Chander and Ahmed, 1989), mammalian anti-fertility (Arora et al., 1971), and insect anti-feedant activities (Chander and Ahmed, 1985)) are found in large quantities.

Several other minor benzoquinones have been isolated from the Myrsinodae (compounds 6, 7, Midiwo et al., 1992a; compound 8, Midiwo and Ghebremeskl, 1993; compounds 10 and 11, Midiwo et al., 1990, compound 12, Midiwo and Arot, 1996). These structures are shown in Figure 2.

Polygonaceae

There are six genera belonging to the Polygonaceae in Kenya (*Emex*, *Harpagocarpus*, *Rumex*, *Polygonum*, *Fagopyrum*, *Oxygonum*) but the initial attraction to the family was the widely used anthelmintic *Rumex* genus. There are five *Rumex* species in Kenya: *R. abyssin*-

Table 2. High concentration benzoquinone pigments in Kenyan Myrsinaceae species (g/kg).

	Embelin/rapanone	Maesaquinone	Acetylmaesaquinone	Maesanin
Maesa lanceolata				
Fruits	_	110.1	5.0	0.41
Root bark	_	26.9	5.1	0.61
Stem bark	_	21.5	0.40	0.01
Leaf	_	13.5	0.45	0.21
Myrsine africana				
Fruits	41.00	_	_	_
Root bark	13.10	_	_	_
Stem bark	14.33	_	_	_
Leaf	18.20	_	_	_
Rapanea melanophloeos				
Fruit	94.70	_	_	_
Root bark	72.70	_	_	_
Stem bark	26.30	_	_	_
Leaf	25.00	_	_	-
Embelia schimperi				
Fruit	43.11	_	_	_
Root bark	10.1	_	_	_
Stem bark	11.2	_	_	_
Leaf	10.7	_	_	_

icus, R. usambarensis, R. bequaertii, R. ruwenzoriensis and R. crispus. Their use in ethno-medicine is shown in Table 3. Morphologically the species can be grouped into sub-genera according to leaf structure; those with hastate (arrow shaped) leaves (R. abysinicus and R. usambarensis) and with oblong lanceolate leaves (R. bequaretii, R. ruwenzoriensis and R. crispus). Analysis of the five species show that the major compounds, especially in roots, which are mostly used in traditional therapy, are the common anthraquinones, emodin (12), physcion (13), and chrysophanol (14), free and glycosidated and the naphthalenic derivative nepodin (15), sometimes referred to as musizin (Midiwo and Rukunga, 1985). It is interesting that the distribution of 15 is discontinuously biased in the oblong-lanceolate leafed species but not in the hastate leafed ones (Table 4) suggesting a sub-division of the genus into sub-genera.

The other genus of the Polygonaceae considered was *Polygonum* which has close resemblance to *Rumex* since they are close in the 'Key to species' and some of its members seem to have similar ethnomedical usage, such as purgative and anti-microbial

Table 3. Traditional uses of Rumex species.

Species	Traditional uses
R. abyssinicus	Leaves and stems are powdered and sap used as treatment for pneumonia and cough. Roots powdered and applied to wounds or infusion drunk as remedy for stomach-ache
R. bequaertii	Roots used for treatment of abscesses, or abdominal pains
R. usambarensis	Leaves used for coughs or ground and mixed with water to stop stomach-ache. Whole plant is used for treatment of smallpox

(Table 5). Only four members of the genus *Polygonum* seem to be applied in ethno-medicine even though there 12 *Polygonum* species in this country – *P. baldschuanicum*, *P. convolvulus*, *P. amphibium*, *P. nepalense*, *P. capitatum*, *P. afromontanum*, *P. aviculare*, *P. strigosum*, *P. salicifolium P. senegalense P. pulchrum and P. setosulum* (Agnew and Agnew, 1994).

RO
OH
$$1 R= H$$
 $2 R = -COCH_3$

HO
$$R$$
 4 R= n-C₁₁H₂₃
OH 5 R= n-C₁₃H₂₇

Figure 1. Major benzoquinones of the Myrsinaceae.

The four that have recorded use are those that appear last on this list which is according to the 'Key to species'. They also have the most widespread distribution. A phytochemical survey of these four led to the conclusion that *P. senegalense* was quite unique and rich in flavonoid secondary metabolites which were actually 'surface exudates'.

P. senegalense is an erect robust perennial which grows up to 3 m tall. It has large petiolate, oblong-lanceolate leaves which reach a size of 28×7 cm. The leaves and stems can be glabrous throughout (P. senegalense forma senegalense) or may be covered by white or yellowish tomentum (P. senegalense forma albomentosum).

Plants with intermediate characteristics are also known. We have even noticed a plant that would have been described as wholly forma *albomentosum* changing rapidly to forma *senegalense*; however, once a leaf is glabrous it never reverts to tomentose form. Either form when pressed deposits yellow coloration on the paper suggesting presence of superficial pigmentation. Indeed there is a surface exudate (which varies in quantity from 3–17% dry leaf weight de-

pending on the age of the leaf-young leaves have higher concentration) which is composed of fifteen non-polar flavonoids – chalcones, dihydrochalcones, flavanones and a flavone (Figure 4) (Midiwo et al., 1990, 1992b). Compound **30** is found only in the exudate of *P. senegalense* forma *senegalense*.

On the other hand, the internal tissue flavonoids are the common flavonoids quercetin (31), kaempferol (32) and luteolin (33) and their glycosides (Midiwo et al., 1994) (Figure 5).

Comparison of Figures 4 and 5 show that the transformation of internal tissue flavonoids to the external exudate ones involves the dehydroxylation of ring B and in some cases an increase in the oxygenation of ring A. This is accompanied by hydroxy group methylation to, ostensibly, maintain the number of hydroxyl groups for the flavonoids at two or less. One exceptional glycoside which was characterised is the 2'- β -O-glucosyl-4'-hydroxyl-6'-methoxydihydrochalcone whose aglycone, uvangolatin (20) is part of the external aerial exudate; these non-polar compounds are biosynthesised in the cellu-

Figure 2. Minor benzoquinones of Myrsinaceae.

lar mileau as usual and then extruded onto the polar surface after hydrolytic removal of the sugar group.

The phenomenon of the existence of external flavonoids found in non polar mixtures (mostly terpenoid) is now firmly established especially through the work of Eckhard Wollenweber (1988) of the University of Darmstadt, Germany. He has observed this character in genera from diverse families: Bignoniaceae (Phyllanthron), Cistaceae (Cistus), Compositae (Achillea, Artemisia Baccharis, Brickelia, Chrysothanamus, Ericameria, Eupatorium, Flourencia, Haplopappus, Heterotheca, Pluchea and Vernonia), Discrastyliceae (Newcastelia), Ericaceae (Gaultheria, Kalmia), Euphorbiaceae (Bayeria), Gesneriaceae (Didymocarpus), Hydrophyllaceae (Eriodictyon, Wigandia) Lamiaceae (Salvia), Mimosaceae (Acacia), Myricaceae (Comptonia, Myrica), Myrtaceae (Eucalyptus), Penaeaceae (Saltera), Primulaceae (Primula), Sapindaceae (Dodonaea), Scrophulariaceae (Mimulus) and Zygophyllaceae (Larrea). Compositae has the greatest genera representation undoubtedly because it is the largest family of flowering plants. A further observation is that the species exhibiting this phenomenon tend to be those from semi-arid or alpine habitats which led to conclusion that this is a character common in plants in xeric habitats or at least plants with such origins. This fact and that most if not all non-polar flavonoids reported are actually accumulated externally in the species from which they are reported is now well established (Wollenweber, 1988). An intriguing question still remains about the function of this phenomenon to the organism; several reasons have been speculated about this such as antifungal, anti-bacterial and anti-viral guards (Wollenweber, 1986). Several workers have also associated the external localisation with importance as insect deterrents such as in *Larrea tridentata* (Rhoades, 1977). The lipophilic flavonoids are assumed to penetrate bet-

Table 4. The distribution of polyketide pigments in Rumex species.

	Nepodi	n	Chryso	phanol	Physcio	on	Emodin	1	
Species	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Total
R. usambarensis									
Roots	_	_	0.263	0.098	0.156	0.073	0.127	0.064	0.078
Seeds	_	_	0.049	0.075	0.036	0.049	0.023	0.086	0.032
Leaves and stems	-	-	0.014	0.034	0.027	Trace	0.013	0.039	0.130
R. abyssinicus									
Roots	-	-	2.070	0.067	1.150	0.030	9.490	0.102	12.91
Seeds	_	_	0.240	0.057	0.290	0.030	0.310	0.078	1.110
Leaves and stems	_	_	0.600	0.025	0.073	Trace	0.069	0.040	0.810
R.bequacertii									
Roots	0.071	0.010	0.089	0.042	0.144	0.029	0.093	0.093	0.570
Seeds	0.046	0.031	0.082	0.063	0.079	0.031	0.089	0.061	0.480
Leaves and stems	0.020	Trace	0.028	Trace	0.032	Trace	0.020	0.060	0.160
R.ruwenzoriensis									
Roots	3.350	0.208	0.319	0.034	0.117	Trace	0.217	0.078	4.320
Seeds	1.170	0.035	0.499	0.154	0.145	0.051	0.126	0.142	2.320
Leaves and stems	0.234	Trace	0.352	0.032	0.048	Trace	0.120	0.036	0.820
R.crispus									
Roots	1.66	0.055	0.933	0.089	0.164	0.055	0.545	0.079	3.580
Seeds	0.498	0.016	0.280	0.031	0.041	0.017	0.164	0.024	1.070
Leaves and stems	0.116	0.004	0.056	0.006	0.008	0.004	0.004	0.038	0.240

Table 5. Traditional uses of Polygonum species (Kokwaro, 1976).

Species	Uses
P. salicifolium	Salt obtained from burned ashes is licked as a cure for sore throat. Leaf decoction is used as a purgative and extract from fresh leaves is used for skin troubles
P. senegalense	Used for removal of ectoparasites from livestock. Also orally administered to cattle for managing unstated diseases
P. setosulum	Used to bath the dying using an infusion of leaves in order to revive them
P. pulchrum	Leaves used for syphilis. The leaf decoction is drunk about three times a day by patient

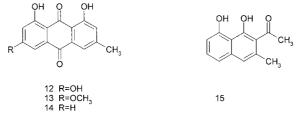


Figure 3. Anthraquinones of Kenyan Rumex species.

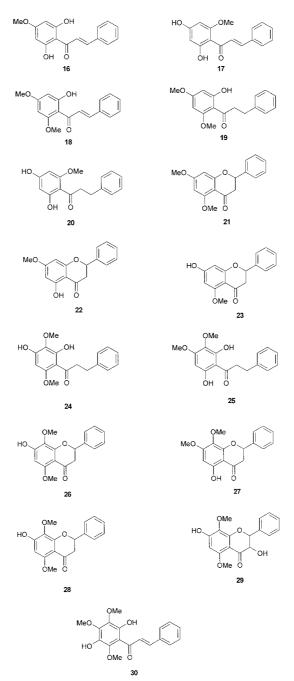


Figure 4. Non-polar flavonoids constituting the aerial surface exudate of P. senegalense.

ter the predator organisms' cell membranes. They have also been assumed to reflect and absorb radiation and thus serving a cooling function for leaves and other aerial plants.

In the case of *P. senegalense* (the first Polygon-aceae reported with surface exudate) we have done the following experiments: the petroleum ether aerial

wash was tested for anti-feedant effect against *Schistocerca gregaria* and *Locusta migrotoria* locusts by the procedure of Butterworth and Morgan (1968) using filter paper and 100% protection was achieved at low concentration (Midiwo et al., 1986) (Table 6).

The exudate and some of its components are larvicidal against *Aedes aegyptii* larvae (Table 7) (Gikonyo,

Figure 5. Internal tissue flavonoids of P. senegalense.

1991) and the exudate and its constituent flavonoids absorb all UVA and UVB of the electromagnetic spectral range (Table 8). These experiments demonstrate clearly that surface exudates play a protective function to organisms that produce them.

That is why the research at our institute got deviated to other Kenyan plants with this character; the work done on one of these plants will be mentioned here.

Psiadia punctulata (DC) Vatke

Psiadia punctulata, Compositae, is an East African plant whose leaf decoction finds a variety of ethnobotanical uses, including treatment of colds, fevers, abdominal pains and for removal of ectoparasites from cattle (Kokwaro, 1976). The shrub is known to be avoided by browsing herbivores like giraffe and goats even during severe drought even though its leaves remain green for a long time as it is quite drought resistant. The leaves of *P. punctulata* have a shiny look

Table 6. Relative Anti-feedant Percentage (RAP) of aerial parts of *P. senegalense* at various concentrations against 5th instar nymphs of *L. migrotoria* and *S. gregaria*.

Locusta migrote	oria			
Conc (µg/ml)	Crude exudate	Chloroform	Ethyl acetate	Quercetin (31)
100.0	100.0	-83.2	-62.2	-92.27
50	100.0	-58.4	-53.9	-67.9
10	100.0	-36.3	-34.3	-57.5
1	64.5	- 6.7	- 2.2	-20.1
Schistocerca gr	egaria			
Conc (µg/ml)	Crude exudate	Chloroform	Ethyl acetate	Quercetin (31)
100	100.0	100.0	-79.6	-89.4
10	100.0	95.6	-19.2	-23.0
1	68.7	86.5	- 7.1	2.9

Table 7. LC_{50} values of P. senegalense components against 2^{nd} instar Aedes aegypti larvae.

		Lc ₅₀ (μg/m	1)
Time (hr)	48	96	168
Surface exudate	11.21	5.75	3.98
2',6'-Dihydroxy-4'-methoxydihydrochalcone (16)	16.15	4.38	2.26
Quercetin (31)	16.15	27.00	8.50

Table 8. Absorption maxima for the surface exudate flavonoids of P. senegalense

Component	Band I (nm)	Band II (nm)
2',4'-Dihydroxy-6'-methoxychalcone (17)	341	298
2'-Hydroxy-4',6'-dimethoxychalcone (19)	336	290
2',5'-Dihydroxy-3',4',6'-trimethoxychalcone (30)	317	_
2',4'-Dihydroxy-3',6'-dimethoxychalcone (24)	344	_
2',6'-Dihydroxy-3',4'-dimethoxychalcone (25)	334	_
2',6'-Dihydroxy-4-methoxydihydrochalcone (16)	316	283
5-Hydroxy-7-methoxy-flavanone (23)	316	285
7-Hydroxy-5,8-dimethoxy-flavanone (22)	320	284
Crude exudate	400-420	
Photosynthesis range	700-600	
UV-A	400-320	
UV-B	320-290	

and develop a sticky feel soon after they are plucked; this is due to the presence of leaf exudate which is found as 24% dry leaf weight. It has a relative antifeedant percent of 97.7% against *L. migrotoria* when applied at a rate of 1000 μ g/ml (Table 9). Its antifungal activity was also not that strong but it mildly

inhibited the coffee berry disease fungus, *Colletotricum coffeanum* and potato rot fungus, *Fusarium oxysporum* by 47.7% and 60.5%, respectively, when mixed with both at 1000 μ g/ml. As opposed to *P. senegalense* exudate, *P. punctulata* crude wash did not show any mosquito larvicidal activity.

Figure 6. Diterpenes and flavones of Psiadia punctulata.

We have isolated and characterised different diterpenoids from the exudate and reported on five of them (Midiwo et al., 1997) and also reported on the five flavonoids present in the exudate mixture (Juma et al.,

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2001) (Figure 5). Psiadia punctulata Vatke has been confused with P. arabica Jaub et Spach and in fact sometimes equated with it such as by Abu Zaid et al. (1991). From our work clear distinction of the two spe-

Figure 6. (Continued)

Table 9. Percentage protection of filter paper by *Psiadia* crude exudate from Locusta feeding.

Concentrations	(μg/ml) Relative anti-feedant percent (RA	P%)
1000	97.7	
100	62.5	
10	51.3	

cies could be made from the fact that *P. punctulata* has fewer flavonoids than *P. arabica* (Abu Zaid, 1991) and it contains both kaurene and trachyloban diterpenoids while *P. arabica* has only kaurene type (El-Dormiaty et al., 1993; Mossa et al., 1992; El-Feraly et al., 1990; Al-Yahya et al., 1987).

Conclusion

Phytochemical work on Kenyan tropical medicinal plants leads to the observation of many potentially pharmacologically active secondary metabolites. The constituents of aerial surface exudate seem to provide both physical protection (sun's rays) and ecological protection against browsers and micro-organisms, a concept that could be of utility in crop preservation.

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