

Phytochemical Evaluation of Some Kenyan Medicinal Plants

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Abstract

There are more than 1200 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 6-methyl group is in accord with morphological sub-family de-limitation. The benzoquinones showed anti-feedant, 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 upto 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'-methoxychalcone whose aglycone, uvangolatin is part of the exudate mixture. Other leaf exudate plants studied included 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 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 along side 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

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 phytochemical 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.

The Myrsinaceae

There are five Myrsinaceae species in Kenya – *Maesa lanceolata*, *Myrsine africana*, *Rapanea melanphloes*, *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 anthelmintic 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.

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

PLANT	USE
<i>M.africana</i>	2-3 Handfuls of fruits are chewed for round worm and tapeworm treatment and remedy for chest pains and stiff joints. No harm is done when too much is taken.
<i>E.schimperi</i>	Fruits chewed as both vermifuge and purgative. Dried fruits and roots are boiled or soaked in water and infusion drunk for intestinal worms.
<i>R.melanphloes</i>	Fruits used as anthelmintic when chewed and eaten in porridge to expel intestinal worms.
<i>M. lanceolata</i>	Fruits used as purgative to remove worms and as remedy for sore throats or eaten to cure tapeworm

Literature search revealed an overflow of reports 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” benzoquinoid. We have also shown that 2,5-dihydroxy-3-undecylbenzoquinone (embelin) (4), 2,5-dihydroxynonadec-14'Z-enyl-benzoquinone (maesaquinone) (1) lower testosterone levels in white New Zealand male rabbits on intramuscular injection (Makawiti and Midiwo, 1991 and Githui et. al., 1991). Embelin is also antifeedant to *Schistocerca gregaria* and larvicidal to *Aedes aegypti* larvae (Midiwo et. al., 1995).

Previously Watt and Breyer-Brandwyk (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. melanphloes*). 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. melanphloes*, *M. africana* and *E. schimperi*) were found to be 2,5-dihydroxydialkyl- and 2,5-dihydroxyalkylbenzoquinone (Tables 2) derivatives, maesaquinone, acetylmaesaquinone, maesanin, embelin, and 2,5-dihydroxybenzoquinone (rapanone), 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* spp) 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 highly 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 [Chander and Ahmed, 1985]) are found in large quantities.

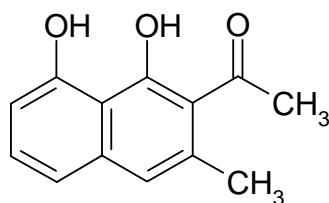
Table 2: High concentration benzoquinone pigments in Kenyan Myrsinaceae species g/kg.

	Embelin/rapanone	Maesaquinone	Acetymaesaquinone	Maesanin
<i>Maesa Lanceolata</i>				
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
<i>Myrsine africana</i>				
Fruits	41.00	-	-	-
Root bark	13.10	-	-	-
Stem bark	14.33	-	-	-
Leaf	18.20	-	-	-
<i>Rapanea melanphloes</i>				
Fruit	94.70	-	-	-
Root bark	72.70	-	-	-
Stem bark	26.30	-	-	-
Leaf	25.00	-	-	-
<i>Embelia schimperi</i>				
Fruit	43.11	-	-	-
Root bark	10.1	-	-	-
Stem bark	11.2	-	-	-
Leaf	10.7	-	-	-

Several other minor benzoquinones have been isolated from the Myrsinodae; 2,3-dihydroxy-5-undecylbenzoquinone, 2-hydroxy-5-methoxy-3-undecylbenzoquinone (Midiwo et al 1992), and 6,6'-bis(2,5-dihydroxy-3-undecylbenzoquinone), 2-hydroxy-3-methyl-6-undecylbenzoquinone (Midiwo and ghebremeskel 1993). Others are from Maesodae; 2,5-dihydroxy-3-pentadec-10'Z-enylbenzoquinone, 2,5-dihydroxy-3-pentadecylbenzoquinone, and 2-methoxy-3-methyl-6-undecylbenzoquinone (Midiwo and Arot 1996).

Polygonaceae

There are six genera belonging to the Polygonaceae in Kenya (*Emex*, *Harpagocarpus*, *Rumex*, *Polygonum*, *Fagopyrum*, *Oxygonum*) but the attraction to it was the widely used anthelmintic *Rumex* genus. There are five *Rumex* species in Kenya: *R. abyssinicus*, *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. abyssinicus* and *R. usambarensis*) and with oblong lanceolata leaves (*R. bequaertii*, *R. ruwenzoriensis* and *R. crispus*). Analysis of the five species show that the major compounds especially in roots, which are mostly used for therapy, are the common anthraquinones (emodin, physcion, and chrysophanol) free and glycosidated and the naphthalenic derivative nepodin (**1**), sometimes referred to as musizin (Midiwo et al 1985). It is interesting that the distribution of **1** 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.



1

Table 3: Traditional uses of *Rumex* species

SPECIES	TRADITIONAL USES
<i>R. abyssinicus</i>	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.
<i>R. bequaertii</i>	Roots used for treatment of abscesses, or abdominal pains
<i>R. usambarensis</i>	Leaves used for coughs or ground and mixed with water to stop stomach-ache. Whole plant is used for treatment of small pox.

TABLE 4: The distribution of polyketide pigments in *Rumex* species, g/kg.

Species	Nepodin		Chrysophanol		Physcion		Emodin		Total
	Free	Bound	Free	Bound	Free	Bound	Free	Bound	
<i>R.usambarensis</i>									
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
<i>R.abysinnicus</i>									
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
<i>R.bequacertii</i>									
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
<i>R.ruwenzoriensis</i>									
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
<i>R.crispus</i>									
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

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 ethno-medical usage, such as purgative and anti-microbiols (Table 5). Only four members of 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.aniculare*, *P.strigosum* *P.salicifolium* *P.senegalense* *P.pulchrum* and *P.setosulum* (Agnew and Agnew 1944).

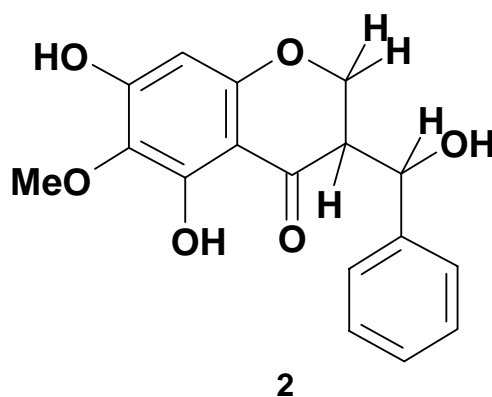
TABLE 5: Traditional uses of *Polygonum* species (Kokwaro,1976)

SPECIES	USES
<i>P. salicifolium</i>	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
<i>P. senegalense</i>	Used for removal of ectoparasites from livestock. Also orally administered to cattle for managing unstated diseases
<i>P. setosulum</i>	Used to bath the dying using an infusion of leaves in order to revive them.
<i>P. pulchrum</i>	Leaves used for syphilis. The leaf decoctions drunk about three times a day by patient.

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. Phytochemical survey of these four led to the conclusion that *P. senegalense* was quite unique and rich in secondary metabolites and attracted our attention to the phenomenon of 'surface exudates'.

P. senegalense is an erect robust perennial which grows upto 3m tall. It has large petiolate, oblong-lanceolate leaves which reach a size of 28 x 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) which is composed of fifteen non-polar flavonoids – chalcones, dihydrachalcones, flavanones and a homo isoflavanone (Midiwo et. al. 1990; 1992). The younger the leaf the more concentrated and the amount of exudate reduces with greater development of the cuticle in older leaves. Compound **2** is found only in exudate of *P. senegalense* forma *senegalense*. The compounds are: 2',6'-dihydroxy-4-methoxychalcone, 2',4'-dihydroxy-6'-methoxychalcone, 2'-hydroxy-4',6'-dimethoxychalcone, 2'-hydroxy-4',6'-dimethoxydihydrochalcone, 2',4'-dihydroxy-6'-methoxydihydrochalcone or uvangolatin, 5,7-dimethoxyflavanone, 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone, 2',4'-dihydroxy-3',6'-dimethoxydihydrochalcone, 2',6'-dihydroxy-4',5'-dimethoxydihydrochalcone, 7-hydroxy-5,8-dimethoxyflavanone, 5-hydroxy-7,8-dimethoxyflavanone, 7-hydroxy-5,8-dimethoxyflavanone, 2',5'-dihydroxy-3',4',5'-trimethoxychalcone and the homoisoflavone (2) of still indeterminate stereochemistry.



On the other hand, the internal tissue flavonoids are the common flavonoids, quercetin, kaempferol and luteolin and their glycosides; the quercetin glycosides characterised are 3 β -O-glycoside, 3 β -O-galactoside, and 3 β -O-(2''-galloylglucoside) (Midiwo et. al. 1994).

Comparison of surface and internal tissue flavonoid forms 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 is part of the external aerial exudate.

The phenomenon of the existence of external flavonoids found in mostly, terpenoid milieu 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*, *Chrysothamnus*, *Ericameria*, *Eupatorium*, *Flourenzia*, *Haplopappus*, *Heterotheca*, *Pluchea* and *Vernonia*), Dipsacaceae (*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 tended to be those from semi-arid or alpine habit 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 remain about the function of this phenomenon to the organism; several reasons have been speculated about this such as anti-fungal, anti-bacterial and anti-viral guards (Wollenweber 1985). 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 better 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 Polygonaceae 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 migratoria* locusts using the procedure of Butterworth and Morgan (1968) using filter paper and 100% protection was achieved at low concentration (Midiwo et al 1986) (Table 6).

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

(a) *Locusta migratoria*

Conc ($\mu\text{g/ml}$)	Crude exudate	Chloroform	Ethyl acetate	Quercetin
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

(b) *Schistocerca gregaria*

Conc (µg/ml)	Crude exudate	Chloroform	Ethyl acetate	Quercetin
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

The exudate and some of its components are larvicidal against *Aedes aegyptii* larvae (Table 7) (Gikonyo et. al., 1998) and the exudate and its constituent flavonoids absorb all UVA and UVB of the electromagnetic spectrum (Table 8). These experiments demonstrate clearly that surface exudates play a protective function to organisms that produce them. The potential of application of *P. senegalense* exudate and those of other plants in development of phyto-pharmaceuticals for human or agricultural use is tremendous.

TABLE 7: LC₅₀ values of *P. senegalense* components against 2nd instar *Aedes aegyptii* larvae.

	Lc ₅₀ (µg/ml)		
Time (Hr)	48	96	168
Surface exudate	11.21	5.75	3.98
2',6'-dihydroxy-4'-methoxychalcone	16.15	4.38	2.26
Quercetin	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	

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 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 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 anti-feedant percent of 97.7% when applied at a rate of 1000 μ g/ml (Table 9). Its anti-fungal activity was also not that strong but it mildly inhibited the coffee berry disease fungus, *Colletotricum coffeanum* and potatoe 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.

We have isolated and characterised different diterpenoids (Fig. 2) 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. 2001); the flavonoids are: 5-hydroxy-7,2',3',4',5'-trimethoxyflavone, 5,7-dihydroxy-2',3',4',5'-tetramethoxyflavone, 5,7,3'-trihydroxy-2',4',5'-trimethoxyflavone, 5,3'-dihydroxy-7,2',4',5'-trimethoxyflavone, 5,4'-dihydroxy-7,2',3',5'-trimethoxyflavone. *Psiadia punctulata* Vatke has been confused with *P. arabica* Jaub et Spach and infact sometimes equated with it such as by Abu Zaid et. al. (1991). From our work clear distinction of the two species 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 and Al-Yahya et. al 1987).

TABLE 9: Percentage Protection of Filter Paper by *Psiadia* Crude Exudate From Locusta Feeding.

CONCENTRATIONS (μ g/ml)	Relative anti-feedant percent (RAP %)
100	97.7
100	62.5
10	51.3

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 protection.

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