The Kenyan higher plant diversity- a potential source of anti-parasitic compounds for neglected tropical diseases.

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Plants and herbology

- Plants have been part of ethno-medicine and therefore ethno-botany since the evolution of man on this planet.
- This process probably started before the advent of human species since it is observed that even primates and other mammals resort to unnaturally chew parts of specific plants when in certain discomforts.
- Indeed the colonization of continents by man, therefore the African continent by the African peoples, is because they could treat their ailments by resorting to herbology to defeat their microscopic invasive enemies.
- Therefore under threat from diseases which are considered neglected, in modern times, Africans should may be re-look at their materia medica of the past for solutions.

Definition according to Wikipedia

- Neglected diseases are a group of infectious diseases that are endemic in the low income populations in the developing world.
- They cause death to about 500,000 to 1m people every year. This death rate is comparable to casualty from HIV/AIDS.
- They are easily preventable but not in affected population areas.

Classification

- They are supposed to be about 13 distinct diseases but can be classified as those caused by:

  -Tryapanosomal parasites

  - Helminth parasites

  - Bacteria and

  - Viruses

Controversy

- The WHO Innovative and Intensified Disease Management (IDM) considers only the following as Neglected tropical diseases (NTDs):
- Buruli ulcer
- Chagas disease
- Cholera
- Human African trypanosomiasis
- Leshmaniasis

More controversy

- These diseases are considered neglected relative to the so called big three- HIV/AIDS, Tuberculosis and Malaria which supposedly receive regular research funding.
- But many individuals and organizations however do not agree.
- DRUGS FOR NEGLECTED DISEASES INITIATIVE (DNDi) organization, considers the following as the neglected diseases:
- African trypanosomiasis,
- Leshmaniasis,
- Chagas disease and
- malaria.
- Thus leaving out Buruli ulcer but including malaria

DNDi and malaria

- DNDi believes that malaria does not get enough attention amongst researchers relative to its perilous effects on populations in tropical areas.
- NDs definition should include “diseases that do not get enough research funding relative to their impact on the population”.

DNDi

- The organisation was formed in 2003 by Medicines Sans Frontieres and five public sector research organisations:
- Kenya Medical Research Institute
- Indian Council of Medical Research
- Malaysian Ministry of Health
- Oswaldo Cruz Organisation of Brazil
- Institut Pasteur of France

DNDi Objectives

- Develop new field –relevant treatments for people suffering from neglected diseases.
- Raise awareness on R&D of drugs for neglected diseases.
- Strengthen research capacity in countries where NDs are endemic.

Phytomedicines for neglected diseases

- Clearly there is folklore (mostly medicinal herbs) about the management of neglected diseases amongst African peoples.
- It would probably be prudent to explore the potential of local herbs for development of PHYTOMEDICINES for local populations for neglected diseases.

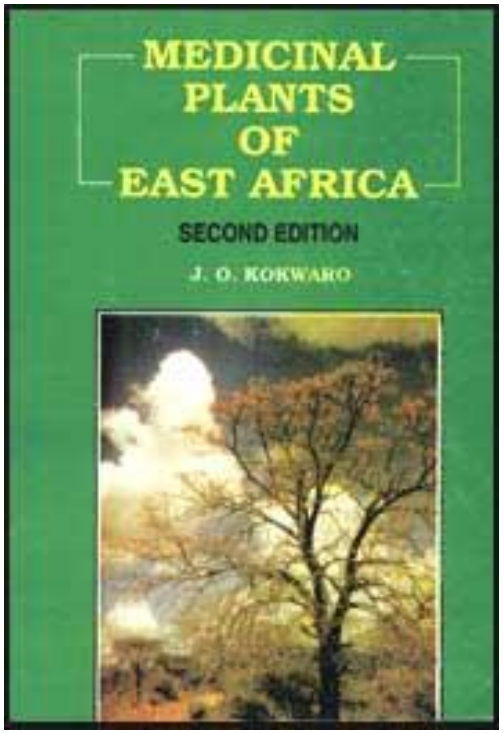
State of Treatment of NDs with medicinal herbs

- Several treatises have been written about the use of plants for treatment of these diseases:
- “Medicinal Plants of East Africa” by John Kokwaro
- “Kenya Trees, Shrubs and Lianas” by Henk Beentje
- etc

“Medicinal Plants of East Africa” by Kokwaro

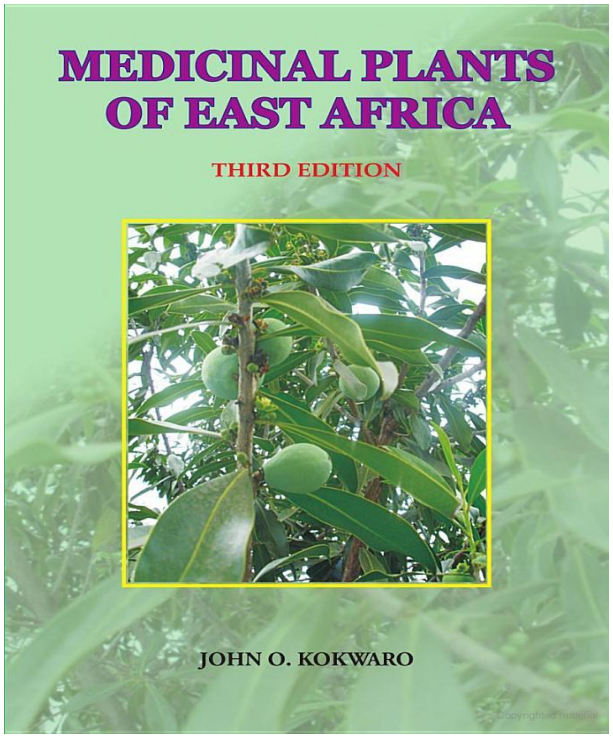
- **Most useful:**
- **Lists plants used for certain disease categories- Helminthiasis, fever including malaria and bacterial infections amongst others.**
- **Describes synoptically the methodology of preparing the herbs from the plants or plant parts**

“Medicinal Plants of East Africa” by Kokwaro



PROF. JOHN O. KOKWARO

Doctor of Science, UoN



SCIRUS for scientific information only

Several hits

Google scholar

‘The Bible’ among medicinal plant researchers

Helminthiasis

- This broad grouping covers many of the NDs
- More than five hundred East African plants are listed by Kokwaro as:
- Emetics/ purgatives,
- Intestinal worm infections alleviators for- hookworm, roundworm, tapeworm, threadworm, general anthelmintics
- Bilharzia and filarial infections

Helminthiasis

- Some plants overlap amongst the categories of helminthiasis by different ethnic groups and should be investigated for their near assured effectiveness.
- We have studied a few such groups of the plants,
- The groups are the Myrsinaceae which are mentioned in several categories of anthelminthiasis and
- *Rumex* species listed as laxatives which can also be used as anthelmintics.

TRADITIONAL USES OF THE MYRSINACEAE

PLANT	*USE*
M. africana	2-3 Handfuls of fruits are chewed for round worm and tapeworm treatment and remedy for chest pains and stiff joints.
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. melanphloes	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 tape worm

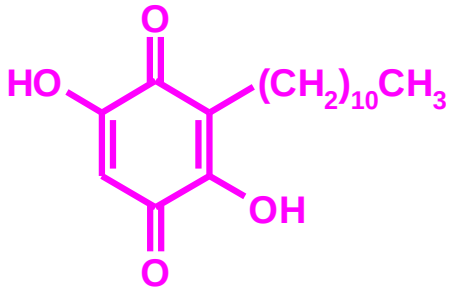
J.O. KOKWARO, 1976; MEDICINAL PLANTS OF EAST AFRICA, EAST AFRICA LITERATURE BUREAU, NAIROBI

Phytochemistry of the Myrsinaceae of Kenya

- The Phytochemistry of the Myrsinacea has been done and corresponds with that already reported in the literature.
- They are habingers of long alkyl side chain 2,5-dihydroxybenzoquinones
- Their chemotaxonomic grouping into two sub-families, the Myrsinodae and the Maesodae coincides with their morphological grouping .

The Myrsinodae

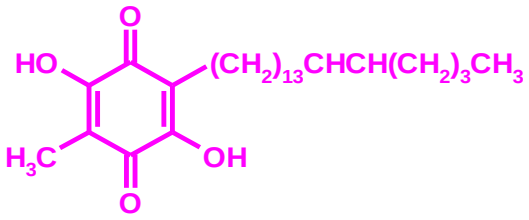
- ***Myrsine africana*,**
- ***Rapanea melanphloes***
- ***Embelia schimperi***
- **They are chemically typified by existence of embelin:**



Embelin

The Maesodae

- ***Maesa lanceolata*** **is the only Maesodae in Kenya. They are typified by the existence of maesaquinone:**



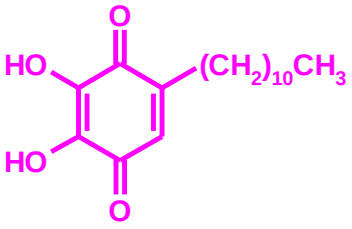
Maesaquinone

TABLE I: BENZOQUINONE PIGMENTS IN KENYAN MYRSINACEAE (g/Kg)

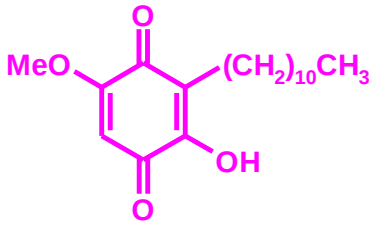
Species	*Embelin/Rapanone*	*Maesaquinone*	*Acetylmaesaquinone*	*Maesanin*
Maesa lanceolata				
Fruits	-	110.1	5.0	0.41
Root bark	-	26.9	5.1	0.65
Stem bark	-	21.5	0.42	0.01
Leaves	-	13.5	0.45	0.21
Myrsine africana				
Fruits	41.0	-	-	-
Root bark	13.0	-	-	-
Stem bark	14.3	-	-	-
Leaves	18.2	-	-	-
Rapanea melanphloes				
Fruits	94.7	-	-	-
Root bark	72.7	-	-	-
Stem bark	26.3	-	-	-
Leaves	25.0	-	-	-
Embelia schimperi				
Fruits	43.1	-	-	-
Root bark	10.1	-	-	-
Stem bark	11.2	-	-	-
Leaves	10.7	-	-	-

J.O. Midiwo, L.M. Arot, C.L. Mbakaya; 1988, *Bull. Chem. Soc. Ethiop*. **2**. pp. 83-85.

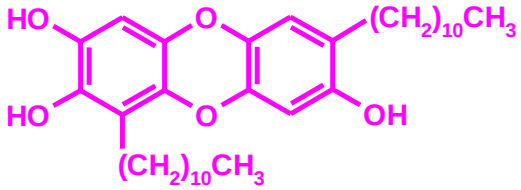
New Myrsinacea benzoquinones



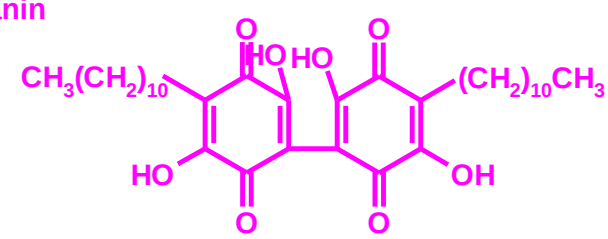
Myrsinone



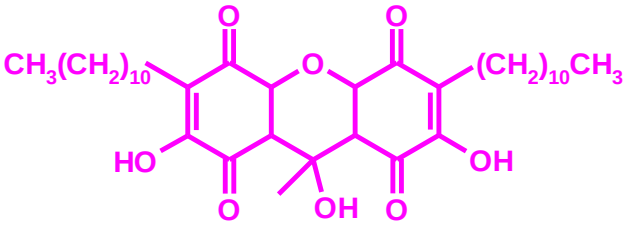
Muketanin



Myrsinaquinone

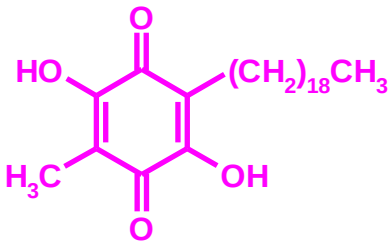


6,6-Biembelin

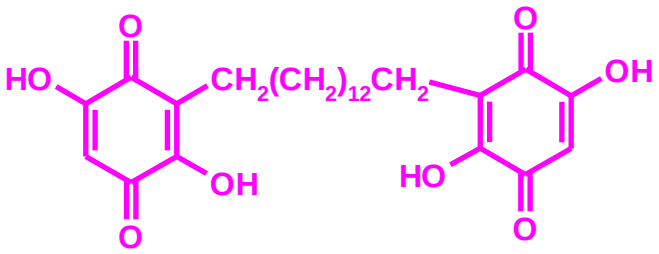


Methylvilangin

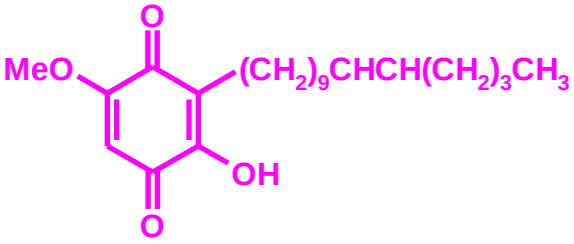
More new benzoquinones



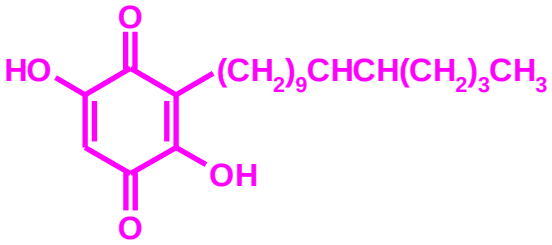
Dihydromaesaquinone



Lanciaquinone



Maesanin

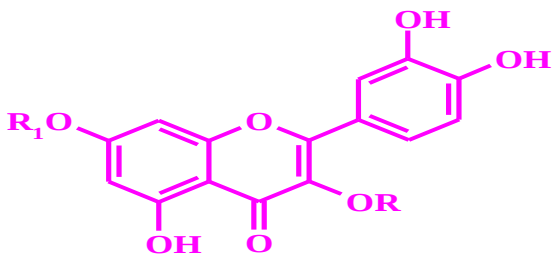


Normaesanin

Benzoquinones in ethno-medicine

- Benzoquinones are known in East africa, India and far east as constituents of folklore medicine against helminths.

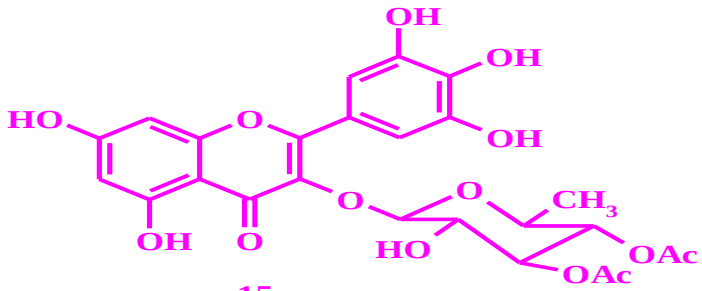
New Flavanoids of the Myrsinaceae



14

R=Rha (4⟶ 1)Glu R1= Glu (6⟶ 1)Rha

L. O. Manguro, J.O. Midiwo, W. Krans Natural Prod. Letters 9, 121-126 (1996)



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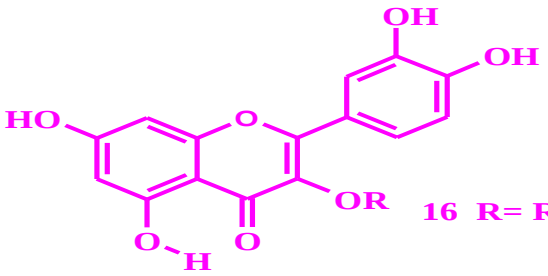
L.O. Manguro, J. O. Midiwo, W. Kraus Phytochemistry, 43(5), 1107-1109 (1996)



16 R= Rha(1⟶ 3)Gal

L.O. Manguro Arot, J.O. Midiwo, W. Krans Natural Prod. Sciences 3(1):8-10 (1997)

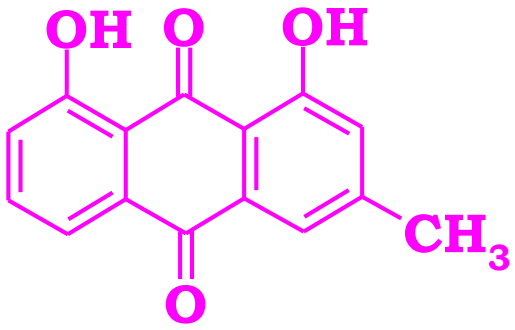
Rumex abyssinicus
Rumex usambarensis
Rumex bequaerttii
Rumex ruwenzoriensis
Rumex crispus

SPECIES	TRADITIONAL USES
R. abysinicus	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. bequaerttii	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 small pox.

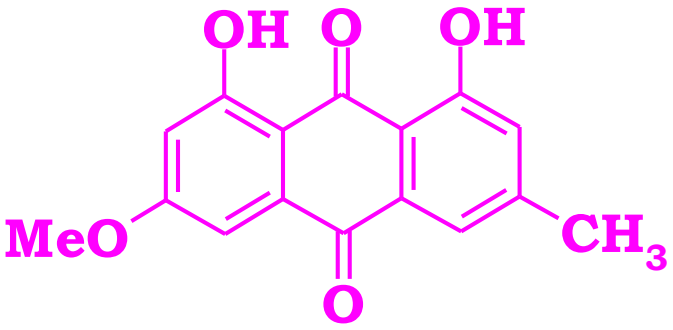
Rumex usambarense

R. abyssinicus with R. usambarense

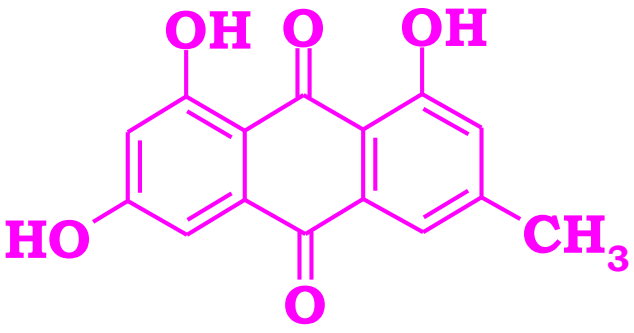
PIGMENTS OF *RUMEX* SPECIES



CHRYSOPHANOL



PHYSCION



EMODIN

NEPODIN OR MUSIZIN

THE DISTRIBUTION OF POLYKETIDE PIGMENTS IN RUMEX SPECIES(% dry weight)

		Nepodin		Chrysophanol		Physcion		Emodin		
SPECIES	**Leaf type**	**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		-	-	0.340	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. bequaerttii	§									
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	0.038	0.006	0.240

Nepodin and the key to species

- **The list in the table above is according to the Key to Species.**
- **Since nepodin is missing in *R. abysinicus* and *R. usambarensis* which have hastate leave but present in the others which have oblong lanceolate leaves, it could be a strong chemotaxonomic marker for sub-genera delienation of *Rumex*.**

Anthraquinones

- Anthraquinones as emodin are established in British and European pharmacopea as anthelmintics

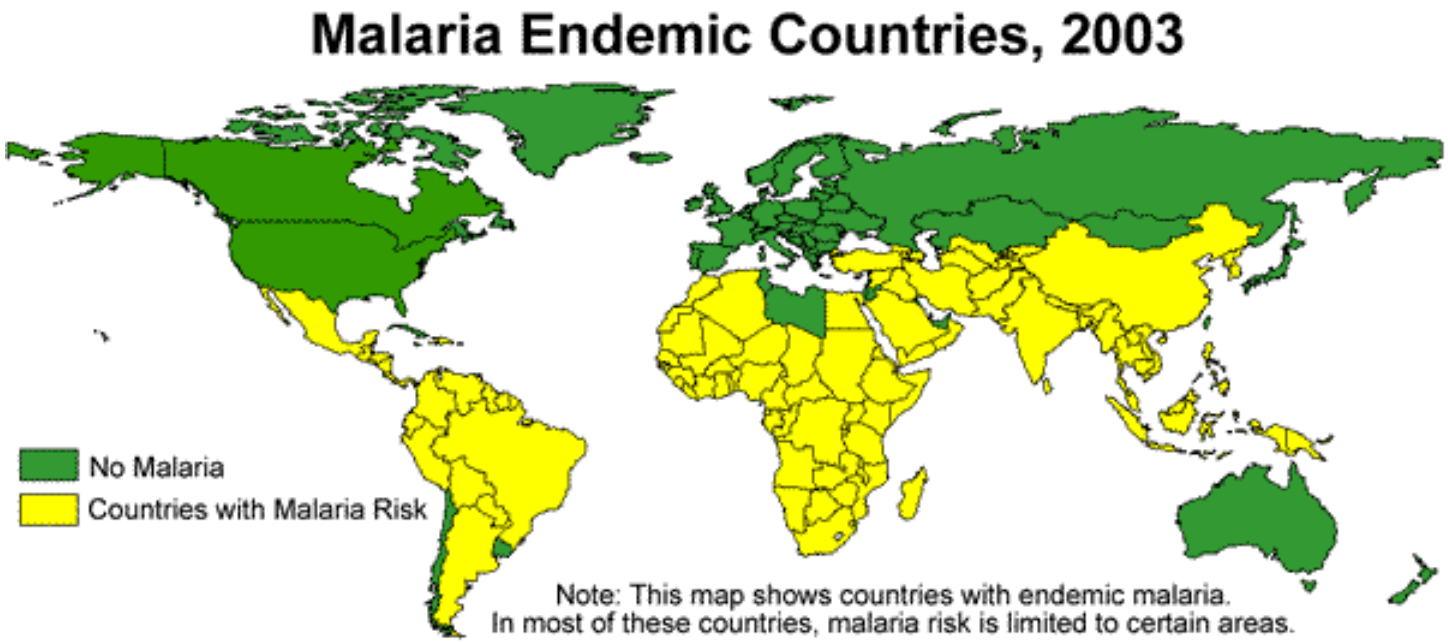
MALARIA

- Malaria is a serious disease whose effects may end in death.
- Symptoms- high fever, chills, flu-like illness.
- Caused by *Plasmodia- P. falciparum* (most common in Africa), *P. ovale*, *P. vivax*, *P. malariae*.
- Illness and death from malaria are largely preventable

MALARIA transmission

- Transmitted by different species of mosquitoes
- The most efficient vector is *Anopheles gambiae* and is the one most prevalent in Africa.

Geographic Distribution:

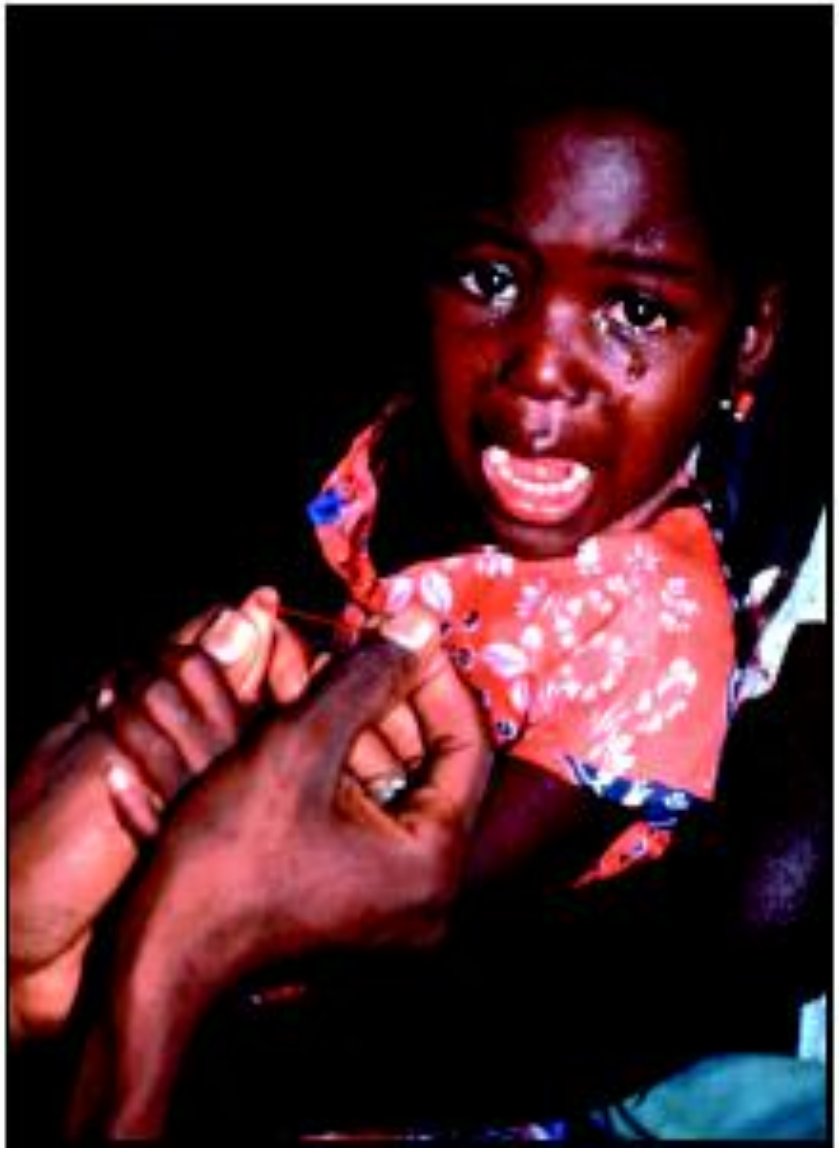


Incidence of Malaria

- 300-500 million infections every year.
- 80% are in Africa.
- 0.5 m deaths every year.
- Malaria constitutes 10% of Africa's total disease burden- 40% health expenditure, and 30-50% of in-patients.
- The total African cost estimate is US$12b.

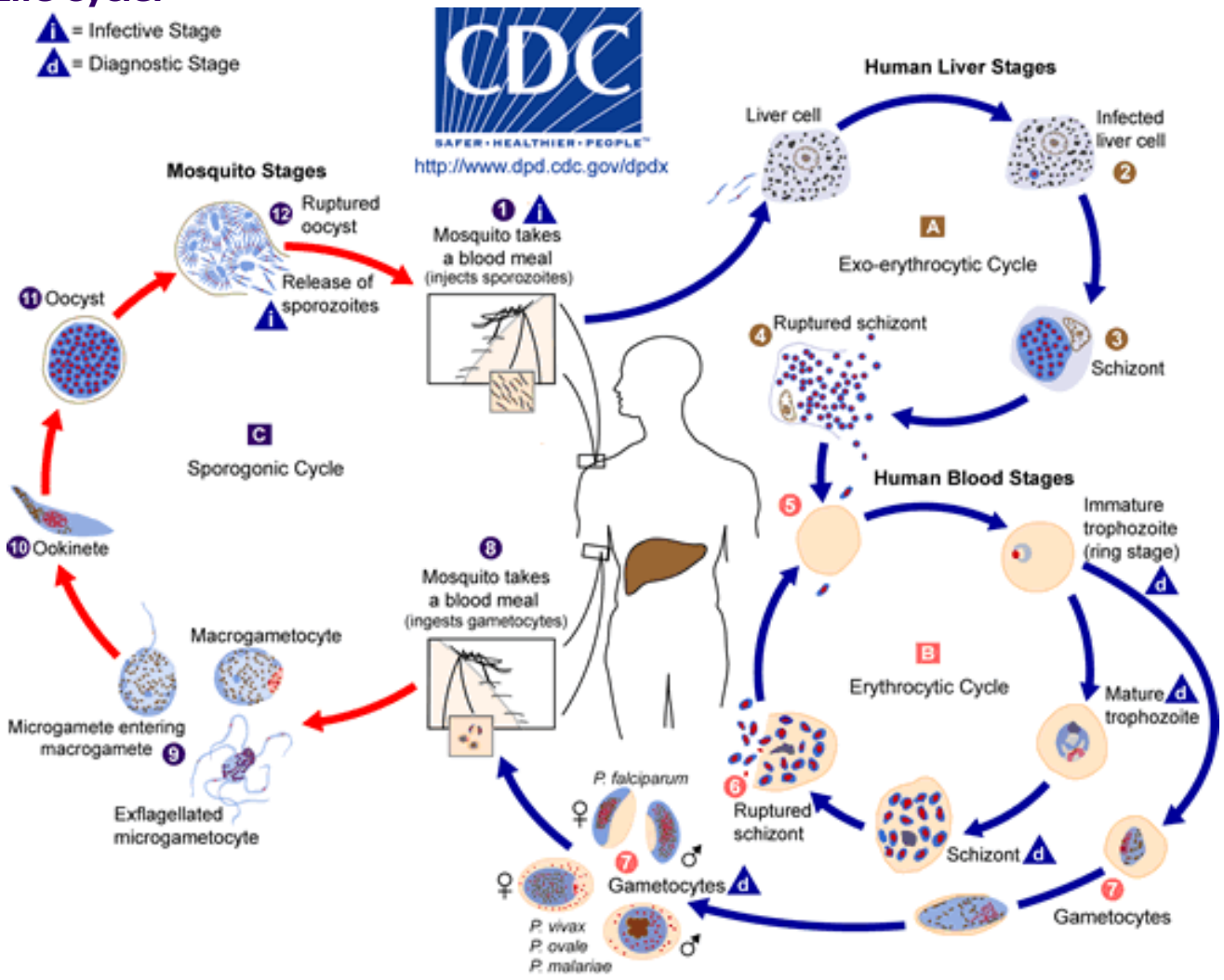
Malaria in Kenya

- **22 million people are at risk, 70% in rural areas.**
- **34,000 children die every year from malaria compared to a total estimate of 42,000 infections.**



75 % Cases

Life Cycle:


i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
Ruptured schizont
4
3
Schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
B
Erythrocytic Cycle
Mature trophozoite
d
6
Ruptured schizont
Schizont
d
7
Gametocytes
P. falciparum
♀
♂
7
Gametocytes
d
P. vivax
P. ovale
P. malariae
8
Mosquito takes a blood meal
(ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10
Ookinete
C
Sporogonic Cycle
11
Oocyst
12
Ruptured oocyst
Release of sporozoites
i
Mosquito Stages
1
i
Mosquito takes a blood meal
(injects sporozoites)

WHY IS MALARIA STILL A PROBLEM?

Anti-malarial drug	Introduced	1st resistance reported	Difference
Quinine	1645	1910	278
Chloroquine	1945	1957	12
Proguanil	1948	1949	1
Sulfadoxine-Pyrimethamine	1967	1967	0
Mefloquine	1977	1982	5
Atovaquone	1996	1996	0

Resistance

Need for Search for anti-malarials

‘Trying to get a solution for malaria is like shooting at a moving target.’

Worse than 20 years ago

Plants as a source of anti-plasmodial principles

- **Many anti-plasmodial compounds are known from plants.**
- **The main examples developed to drug stage are: Quinine from *Cinchona* from South America.**
- **Artemisinin from *Artemisia annua* from China**
- **Different cultures from around the world have used several other herbs for plasmodial disease control.**
- **African malaria ethno-medicines could yield effective anti-malarials as well.**
- **Natural compounds from non-ethnomedically re-known antimalarial plants may also be anti-plasmodial- so need for generalised bioassay survey of plant extracts.**

What are ACTs?

- ACTs stands for artemisinin combination therapies.
- Artemisinin or derivative is constituted into drugs in combination with lumefantrine, mefloquin or sulfadoxin/ pyrimethamine.
- This is for the reason to circumvent resistance development and to improve drug pharmacokinetic properties (It has a short blood circulation lifetime and patients are prone to re-infection due to recrudescence).

Herbal Therapy suggestion

- The use of improved herbals should be encouraged
- An infusion of 5g of *A. annua* leaves with 1l of water taken four times a day for 5 days gets rid of all parasitemia.
- Instead of using ARTEMISININ, use *Artemisia annua* powder or extract in tablet or capsule form.
- *Artemisia annua* leaf has 29 sesquiterpenes (including artemisinin) and 36 flavonoids many of which have or potentiate anti-plasmodial activity- SYNERGISM.
- Use of pure artemisinin, even in combination with one or two drugs will likely lead to resistance development, which is already being observed in parts of Thailand.

HCTs- proposal

- To enhance activity and circumvent resistance development completely, *Artemisia annua* extract or artemisinin could be formulated with other herbals so as to create herbal combination therapies (HCTs). This akin to practice in traditional medicine.
- Many Kenyan plants are known in ethno-medicine for use as anti-malarials.
- Some have been tested and proved to be active.

ANTI-PLASMODIAL FLAVONOIDS FROM THE STEM AND ROOT BARK OF *ERYTHRINA ABYSSINICA*

ERYTHRINA ABYSSINICA

Bark eaten by the Mahale Chimpanzees (Tanzania).

E. abyssinica **is used 'also' by humans to treat malaria.**

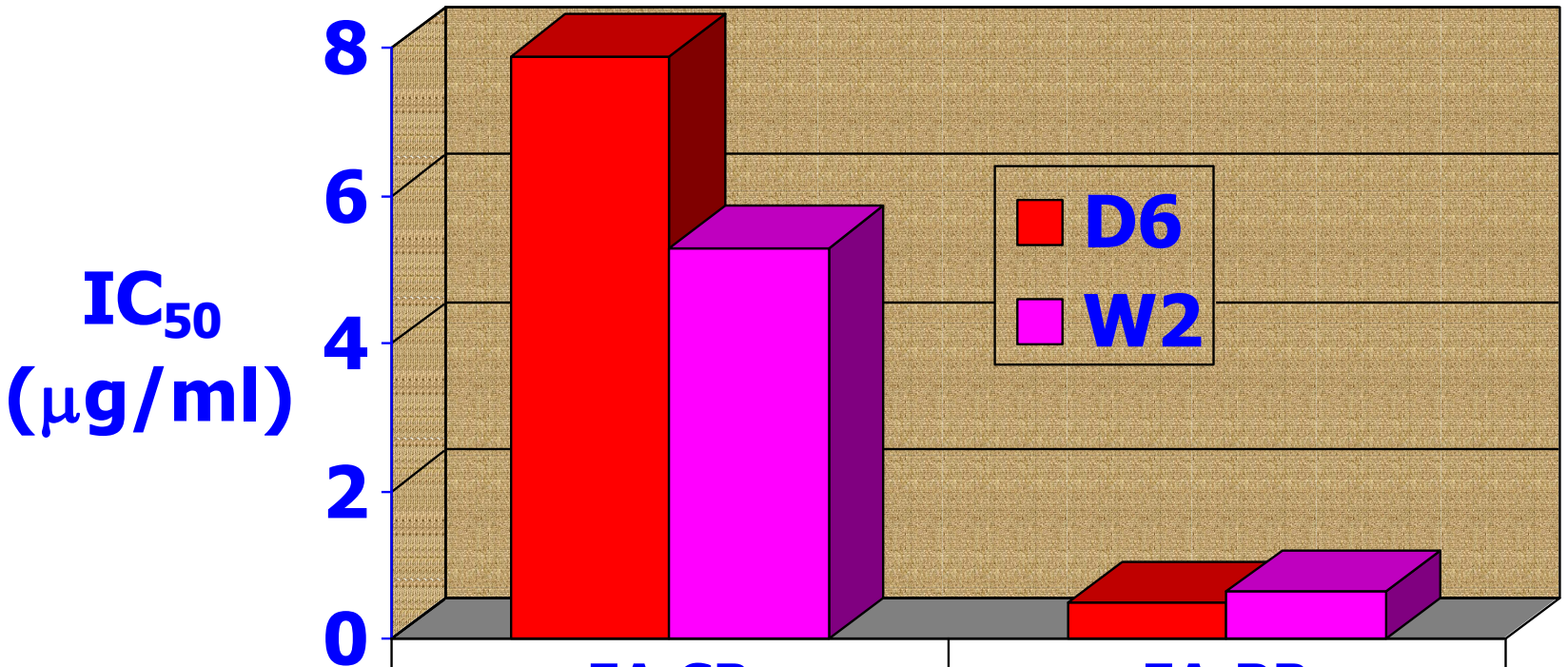
ANTIPLASMODIAL TEST

Test done on two different strains of *P. falciparum* KEMRI

D6 (Chloroquine sensitive)

W2 (Chloroquine resistant)

IN VITRO ANTI-PLASMODIAL TEST RESULTS ON *E. ABYSSINICA* CRUDE EXTRACTS



	EA-SB	EA-RB
D6	7.9	0.49
W2	5.3	0.64

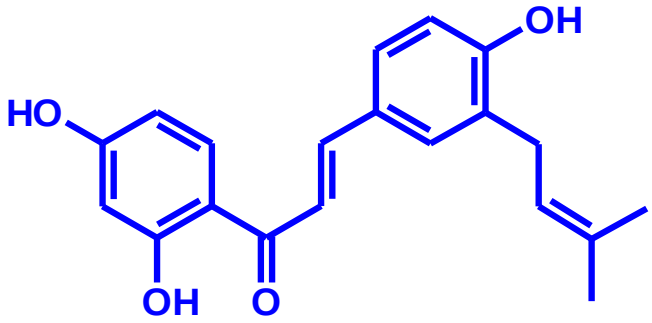
Observe

- That the crude extract mixtures are quite effective especially the root bark which is the preferred remedy.
- In most cases crude extracts are more active than individual single compounds.
- This is attributed to mixture of compounds acting at different centres of the target and synergistcally overcoming the organism.

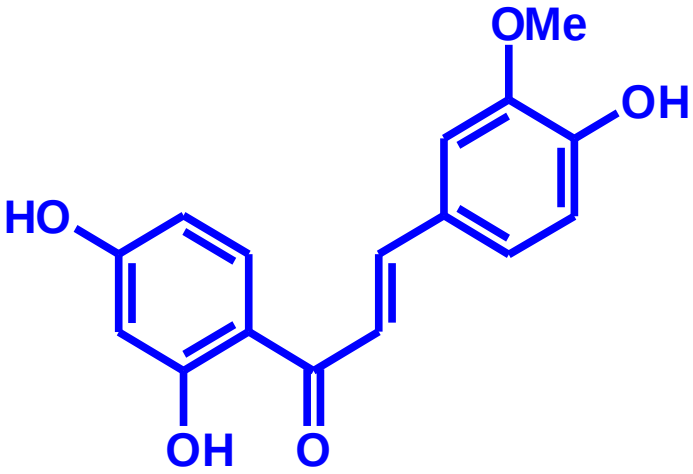
SUMMARY OF THE FLAVONOIDS ISOLATED FROM THE STEM AND ROOT BARK OF *E. ABYSSINICA*

- Three chalcones (1 new),
- 11 Prenylated flavanones (1 new),
- 3 Pterocarpanoids (1 new) and
- 1 New Isoflav-3-ene

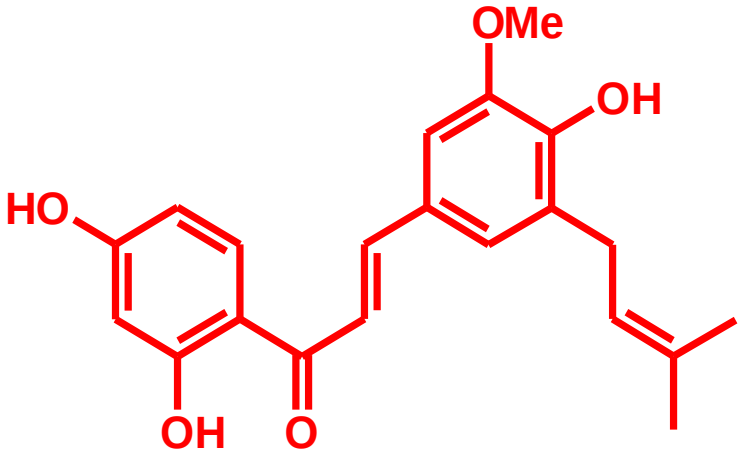
ANTI-PLASMODIAL CHALCONES



IC_{50} = 3.8 ug/ml (D6)
= 3.1 ug/ml (W2)

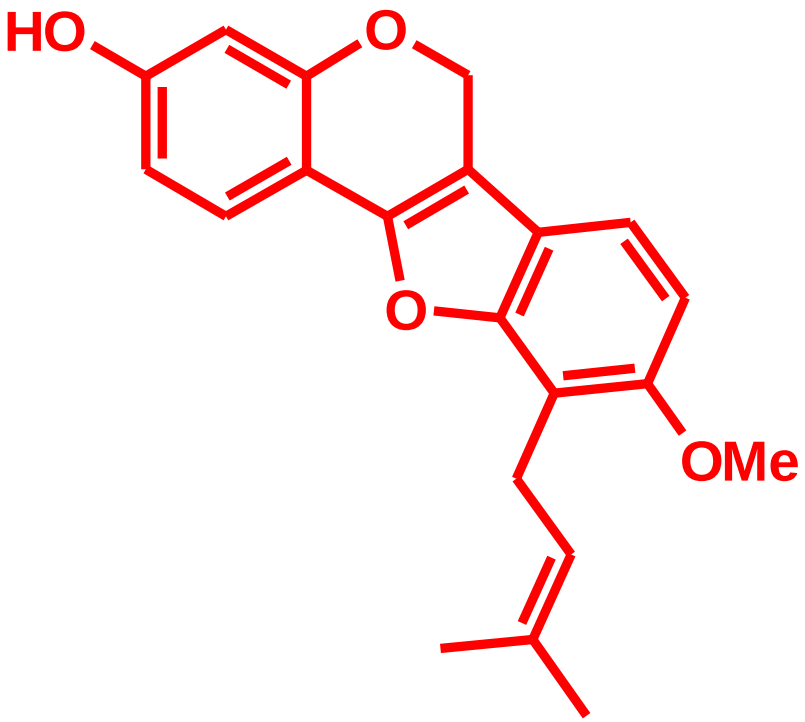


IC_{50} = 4.2ug/ml (D6)
= 4.6ug/ml (W2)



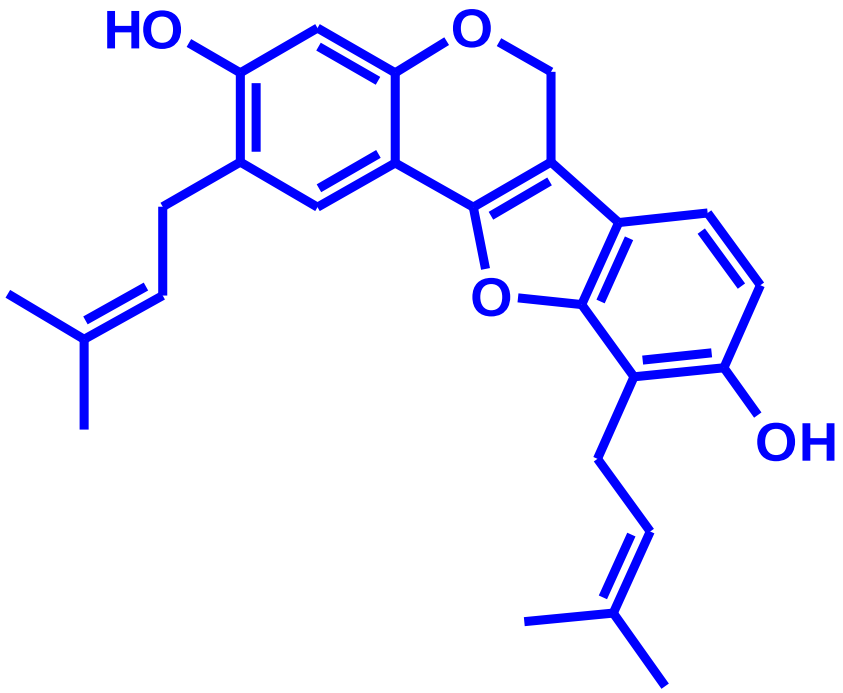
IC_{50} = 3.0ug/ml(D6)
= 3.2 ug/ml(W2)

Anti-plasomodial Pterocarpanoids



3-Hydroxy-9-methoxy-10-prenylpterocarpene

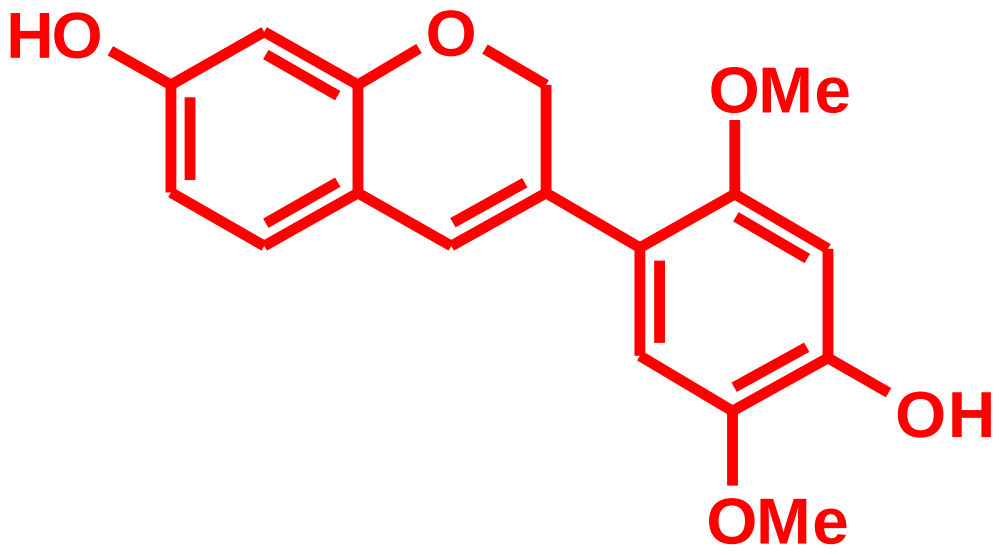
IC_{50} = 5.3 ug/ml (D6)
= 5.1 ug/ml(W2)



Erycristagallin

IC_{50} = 4.9 ug/ml (D6)
= 5.0 ug/ml (W2)

Anti-plasmodial isoflav-3-ene



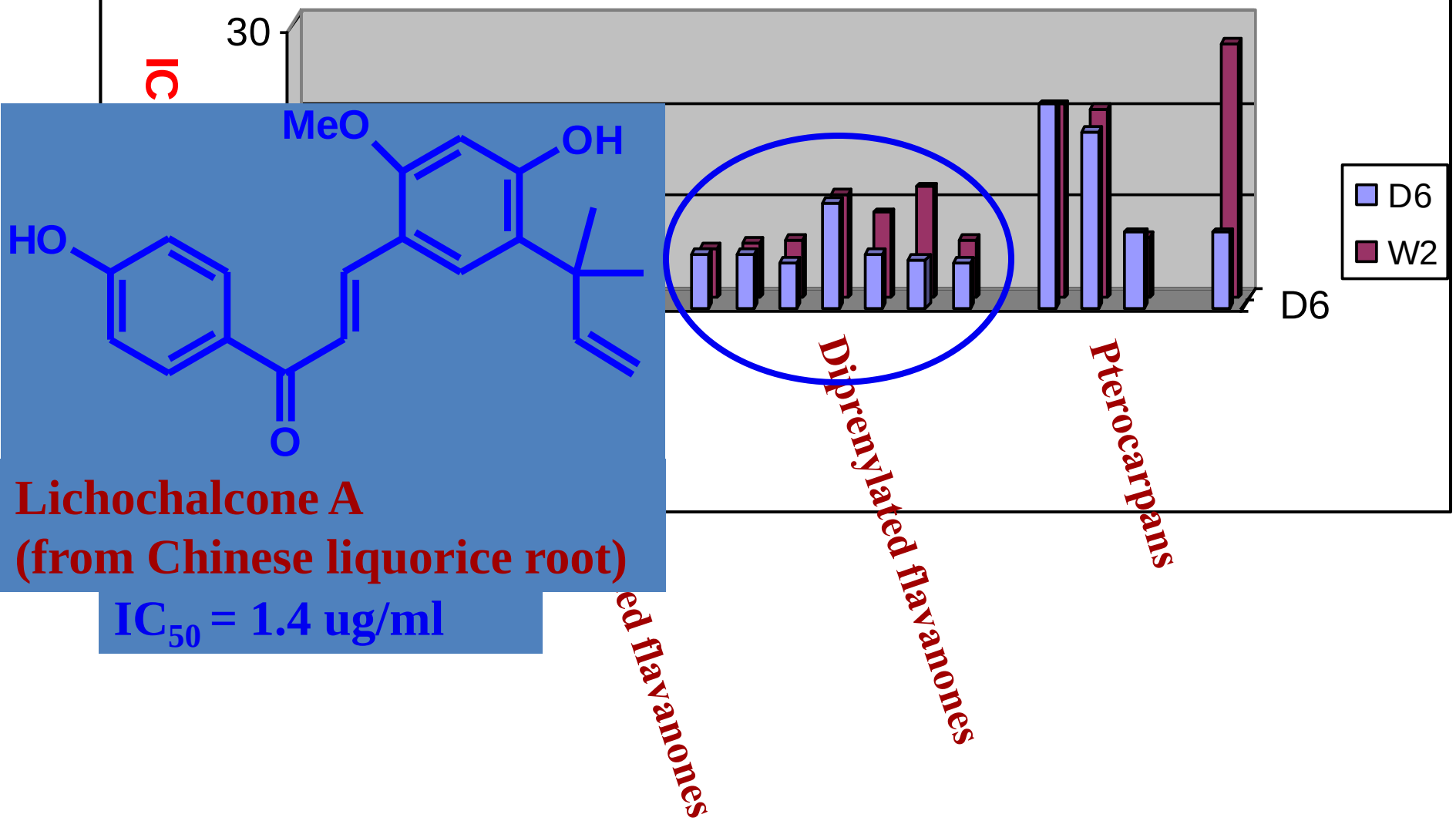
7,4′-Dihydroxy-2′,5′-dimethoxyisoflav-3-ene

IC_{50} = 2.0 ug/ml (D6)

= 6.9 ug/ml (W2)

TO SUMMARIZE

Comparison of the anti-plasmodial activities of the flavonoids of *Erythrina abyssinica*



Lichochalcone A
(from Chinese liquorice root)

IC_{50} = 1.4 ug/ml

Collaboration since 2008

Established bioassay/spectroscopy center .

- **University of Mississippi, School of Pharmacy , Center of Natural Products Research.**

Larry Walker

Muhammad Ilias

Bioactivity approach to compounds for tropical diseases

- Wide collection of medicinal and related plants in Kenya in small quantities of 100- 200 g.
- Make organic solvent extracts and test them *in vitro* against pathogenic protozoa and the microorganisms.
- These tests are done in collaboration at the School of Pharmacy, University of Mississippi in the USA.
- Active plant parts are collected in larger quantities, for compound isolation and further testing for activity, selectivity and cytotoxicity testing.
- *In vivo* testing is then considered.

Preliminary results with some Kenyan medicinal plants with D6 clone of *Plasmodium falciparum*

140 Organic solvent plant extract samples tested at 15.9 ug/ml. Those with less than 50% inhibition are considered not active.

Plant name	Family	% Inhibition
Microglossa pyrifolia	Compositae	79
Sphaeranthus bullatus	Compositae	78
Albizia gummifera	Leguminosae	94
Albizia schimperiana	Leguminosae	98
Erythrina sacleuxii	Leguminosae	60
Erythrina burtii	Leguminosae	73
Erythrina abysinica	Leguminosae	98
Zanthoxyllum holtzianum	Rutaceae	73
Terminalia brownii	Combretaceae	61
Triclisia sacleuxii	Menispermaceae	94
Suregada procera	Euphorbiaceae	68

Roots of *Clerodendrum eriophyllum* (Verbenaceae)

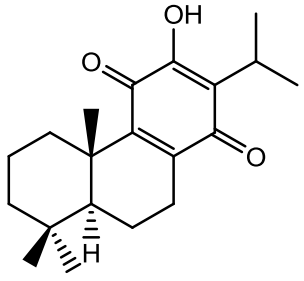
Shrub/small tree, 0.5 - 2 m high, scattered in the dry bushlands of Eastern Kenya

IC_{50} 10 µg/ml for crude extract.

Compounds isolated

- Ten abietane diterpenoids
- One long chain ester of tyrosol ester
- One steroid

Compounds from the roots of C. eriophyllum



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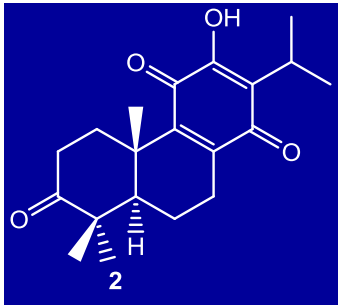

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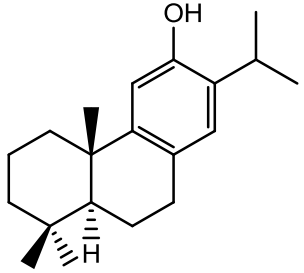

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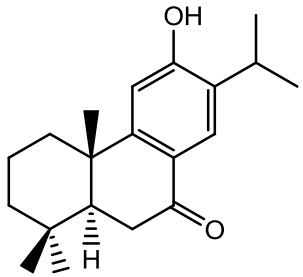

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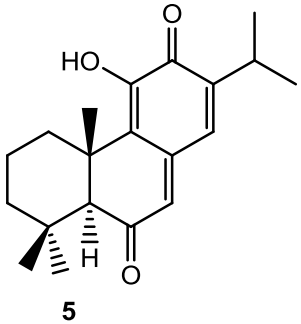

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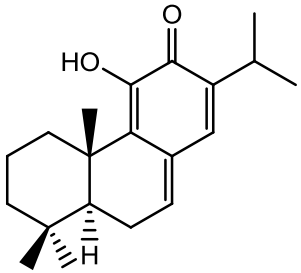

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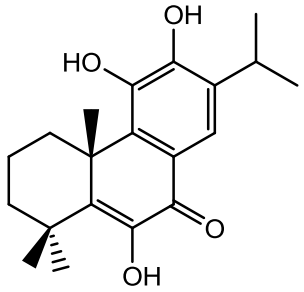

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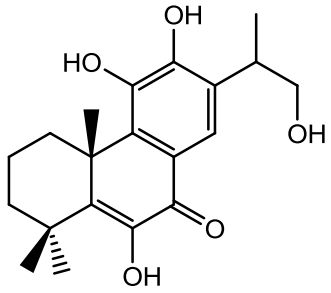

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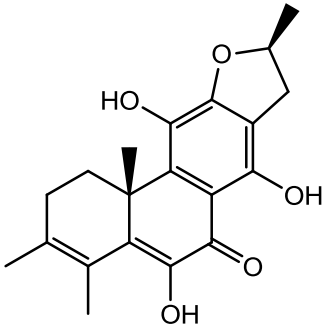

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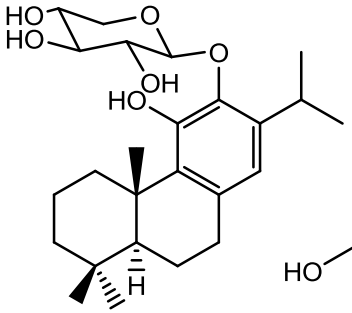

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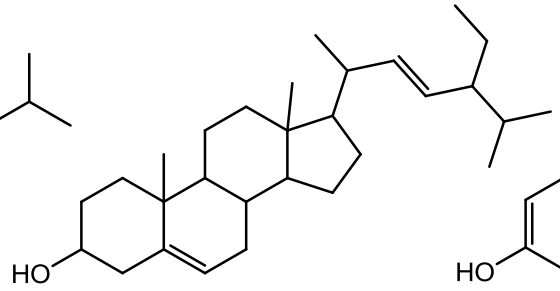

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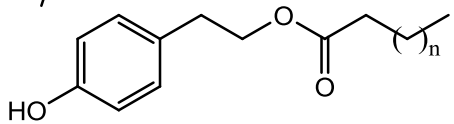


12

Anti-plasmodial activities of *Clerodendrum eriophylum* compounds

Compound/extract	*P. falciparum*		VERO	*L. donovani*	
	D6[a]	W2[b]	TC_{50}	IC_{50}	IC_{90}
	IC_{50}, μg/mL		μg/mL	μg/mL	μg/mL
C.eriophyllum extract	8.8	8.8	NC	NT	NT
1	-	-	NC	16	32
2	-	-	NC	NT	NT
3	1.2	1.2	NC	0.08	0.21
6	-	-	NC	4	13
7	1.8	2.5	4.5	3.2	6.5
8	3.0	4.8	NC	12	22
9	-	-	NC	0.2	0.9
10	-	-	NC	NT	NT
Chloroquine	<0.026	0.14	NC	NT	NT
Artemisinin	<0.026	<0.026	NC	NT	NT
Pentamidine	NT	NT	NT	1.4	6
Amphotericin B	NT	NT	NT	0.13	0.3

Sphaeranthus bullatus (Asteraceae)

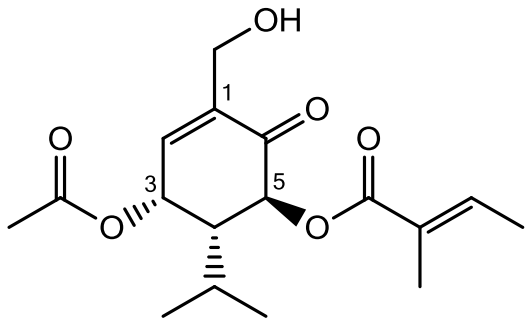
A shrub distributed in tropical and subtropical areas of Africa, Asia and Australia

IC_{50} D6 9.7 W2 15.0 ug/ml

Compounds isolated (aerial parts)

- Four flavonoids
- Six *O*-substituted monoterpenes
- Four Carvotacetones
- One caffeic acid

Compounds from the aerial parts of *S. bullatus*



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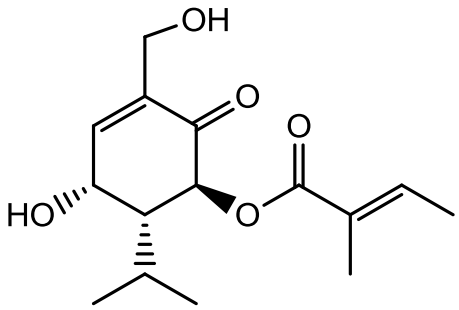

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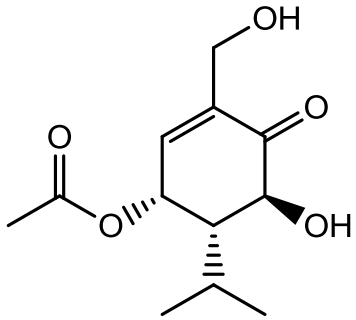

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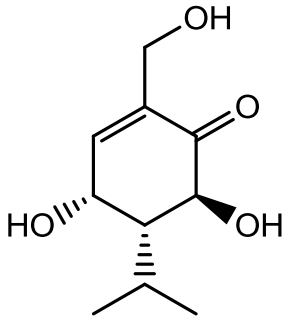


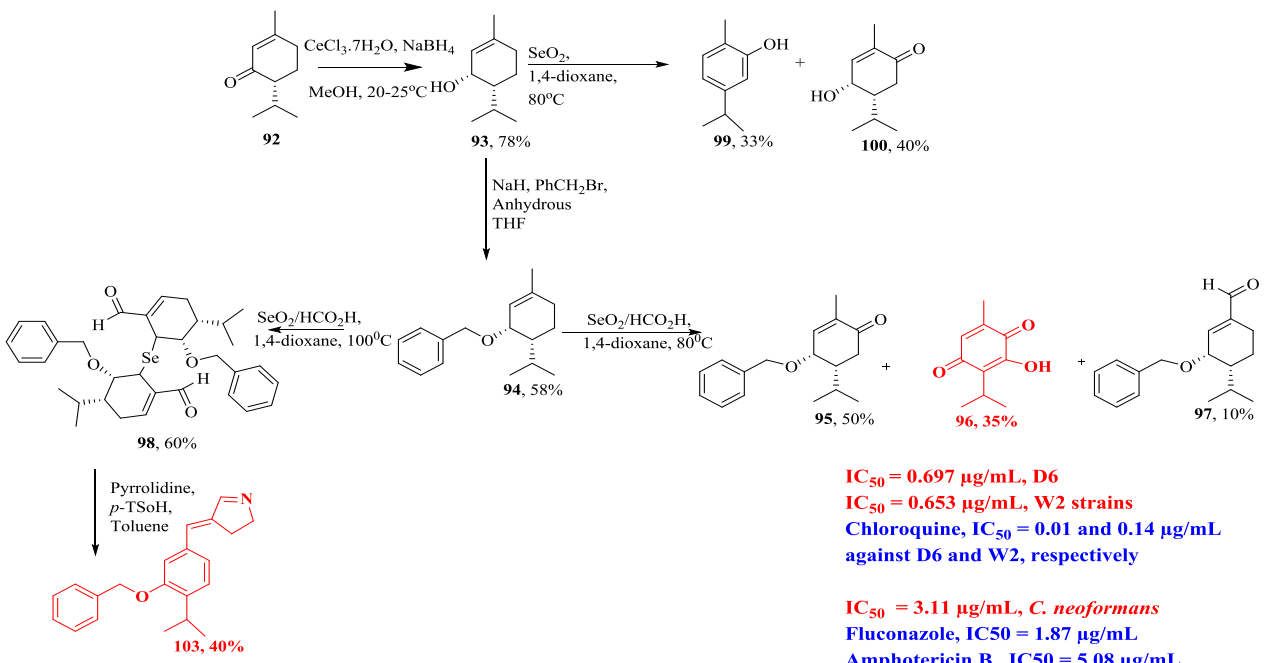
4

Anti-plasmodial and anti-Leshmainal activities of *Sphaeranthus* carvotacetones

	P. falciparum				Vero	L. donovanii	
	D6 (µg/mL)		W2 (µg/mL)		µg/mL	µg/mL	
	IC_{50}	SI	IC_{50}	SI	TC_{50}	IC_{50}	IC_{90}
Extract	9.70	NT	15.0	NT	NT	NT	NT
1	1.40	2.0	2.00	1.4	2.80	0.70	1.30
2	0.79	>6.2	0.90	>5.3	NC	3.00	6.90
3	0.60	21.7	0.68	19.1	0.013	0.70	1.40
4	3.40	>1.4	2.80	>1.7	NC	17.0	>40
Chloroquine	0.01	NT	0.14	NT	NT	NT	NT
Artemisinin	0.004	NT	0.005	NT	NT	NT	NT
Pentamidine	NT	NT	NT	NT	NT	0.1	NT

NC = Not Cytotoxic; NT = Not Tested, IC_{50} = concentration that affords 50% inhibition of growth, SI = Selectivity index

Synthetic scheme for the preparation of carvotacetone analogues



IC_{50} = 0.306 µg/mL, D6
IC_{50} = 0.165 µg/mL, W2 strains
Chloroquine, IC_{50} = 0.01 and 0.14 µg/mL against D6 and W2, respectively

IC_{50} = 3.098 µg/mL, *C. neoformans*
Fluconazole, IC50 = 1.87 µg/mL
Amphotericin B, IC50 = 5.08 µg/mL

IC_{50} = 0.697 µg/mL, D6
IC_{50} = 0.653 µg/mL, W2 strains
Chloroquine, IC_{50} = 0.01 and 0.14 µg/mL against D6 and W2, respectively

IC_{50} = 3.11 µg/mL, *C. neoformans*
Fluconazole, IC50 = 1.87 µg/mL
Amphotericin B, IC50 = 5.08 µg/mL

Plants chosen from the list

- ***Microglossa pyrifolia***
- ***Abrus schimperi***
- ***Dodonaea angustifolia***
- ***Croton alienus***
- ***Clerodendrum eriophyllum***
- ***Sphaeranthus bullatus***
- ***Albizia schimperiana***
- ***Terminalia brownii***
- ***Suregada zanzibariensis***
- ***Suregada procera***

Conclusions

- African traditional herbs can be improved by Phytochemistry through formulating the set of secondary metabolites as a combination.
- The concentration of mixtures can be developed starting from the natural ratios.
- The approach will create more effective , less toxic drugs which are less prone to immune development by microorganisms.

Acknowledgements

- I would like to acknowledge all those students who have travelled with me in research and who have gone on to establish themselves at this University and elsewhere.
- Funding agencies who have contributed to our research funds- Currently, ISP of Uppsala University.
- University of Mississippi colleagues are also acknowledged for their collaboration.

Gratitude

- **Thank you to the committee for giving me a chance to give the lecture and to you the audience.**