Discovering multiple novel magic bullets towards combinatorial cure of malaria without the shadow of resistance

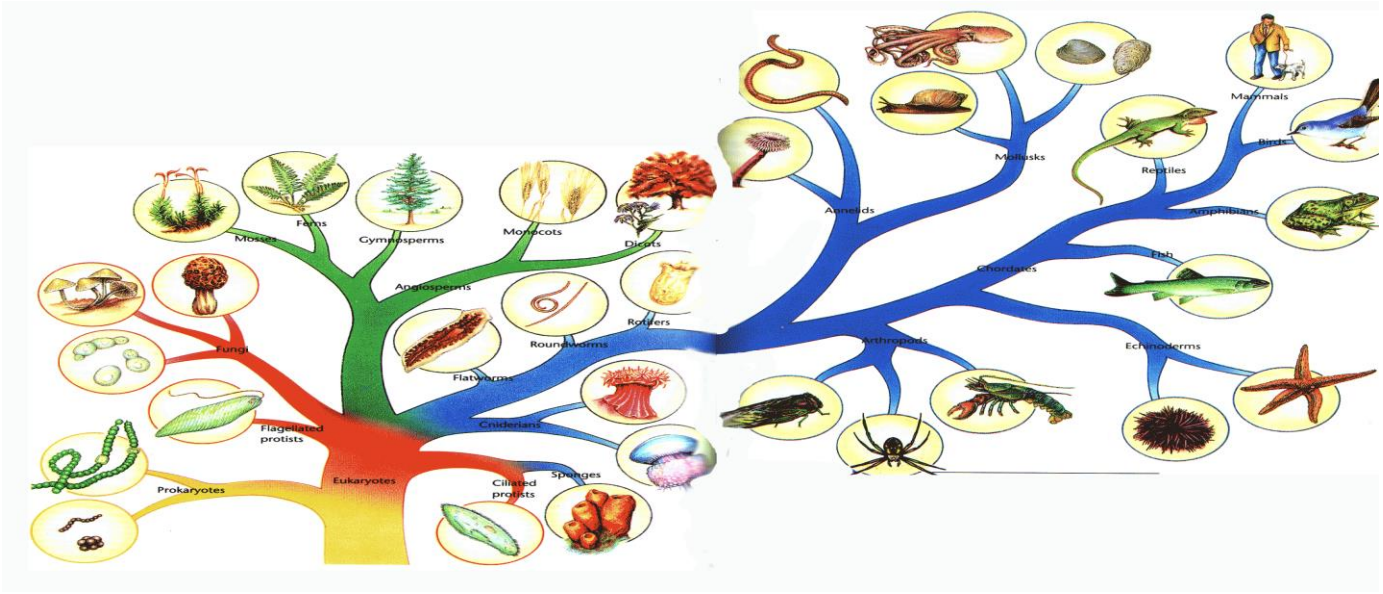
Dinkar Sahal

Malaria Research Laboratory
International Centre for Genetic Engineering and Biotechnology (ICGEB)
New Delhi
dinkar@icgeb.res.in

Malaria parasite is more ancient than we are

What is the nature of malaria parasite that makes it what it is : both ancient and modern at the same time?

Malaria Parasite has evolved ecclectically & has ventured to Parasitize distant life forms



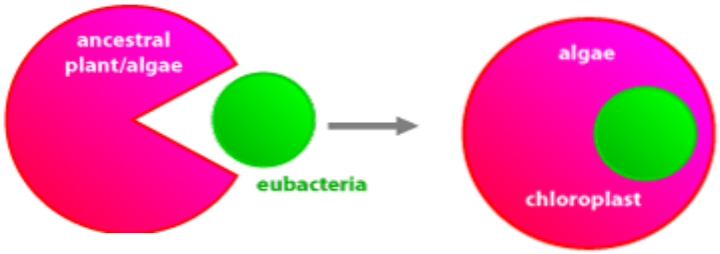


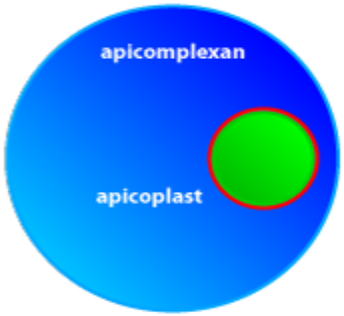


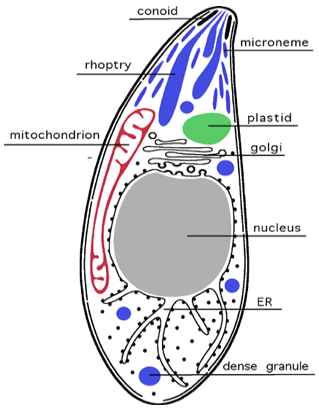




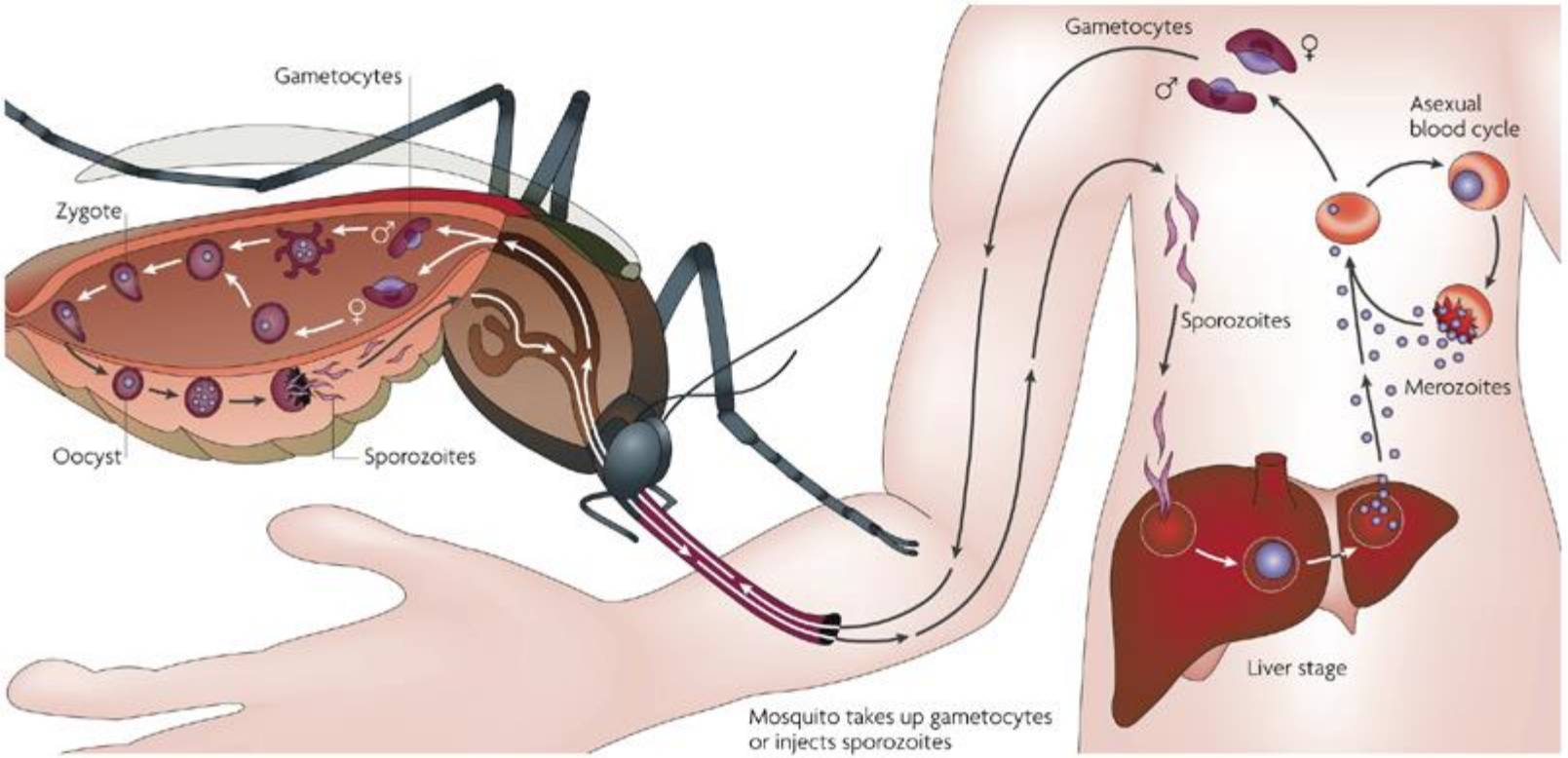








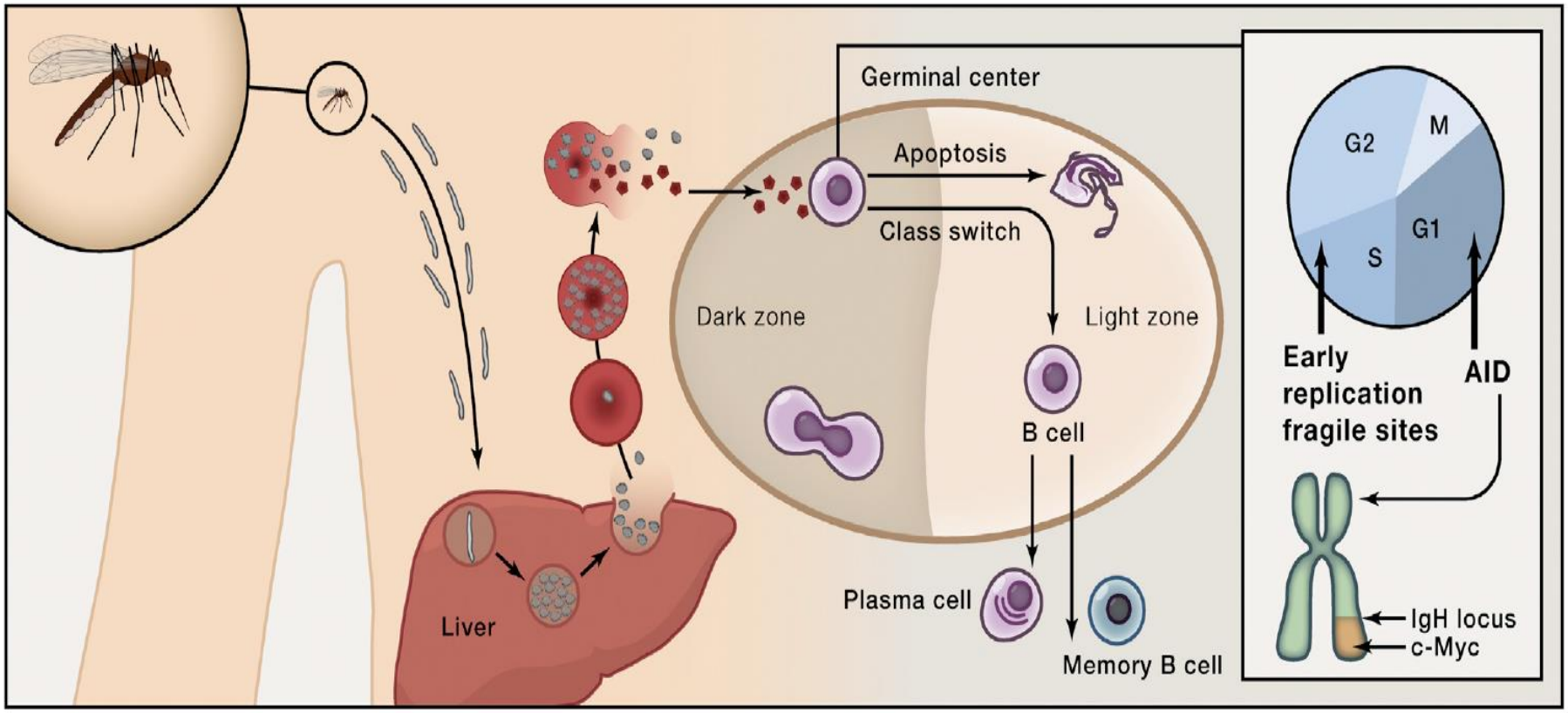
Malaria Parasite cycles across mosquito salivary gland to our liver first & our blood next



***Plasmodium falciparum* life cycle**

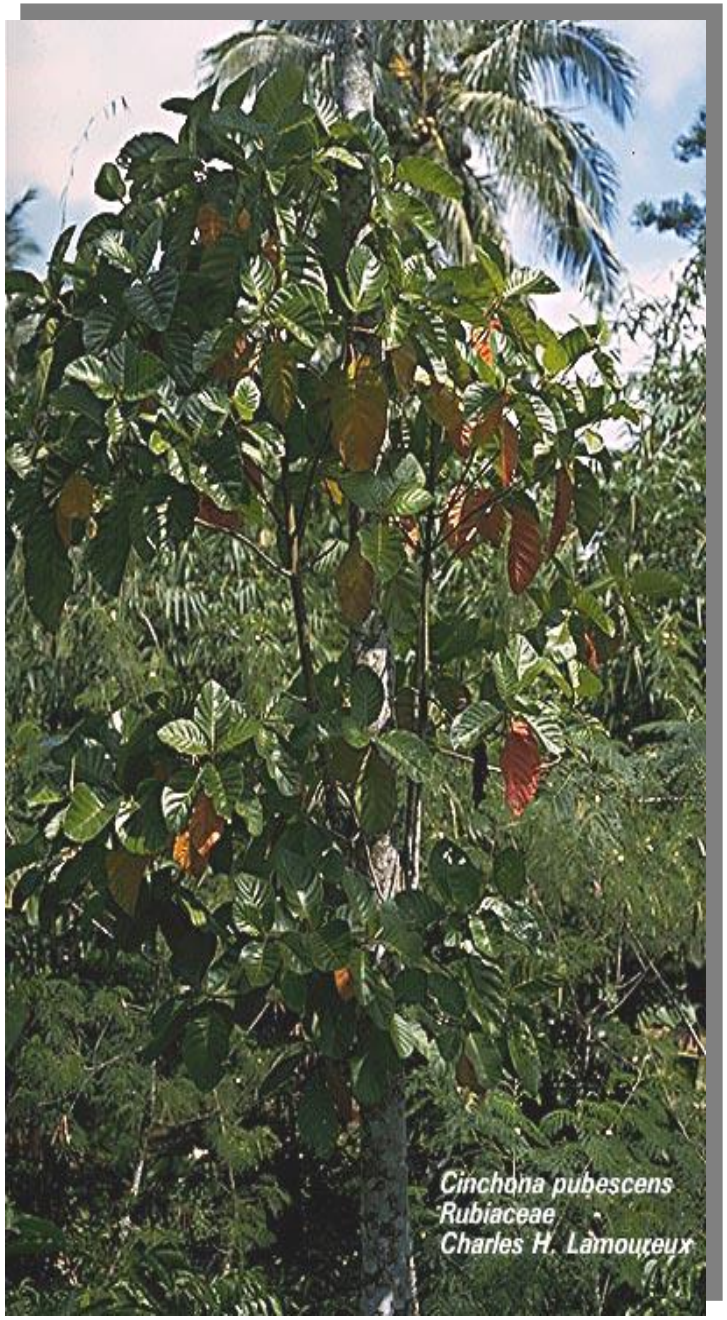
Sinister Repercussions of Malaria:

Malaria-Induced B Cell Genomic Instability

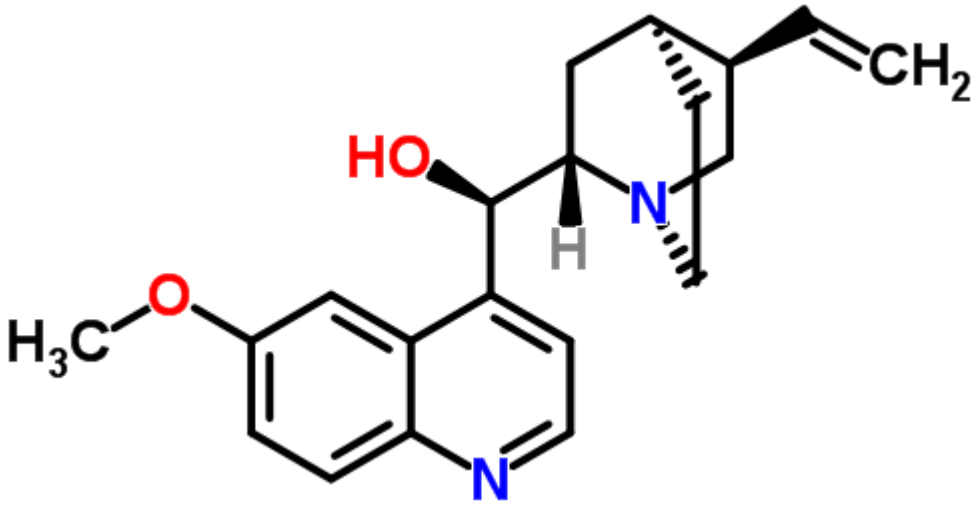


From Mosquito to Lymphoma

Gerson Rothschild et al, Cell 162, August 13, 2015



Long before we knew anything about Malaria the Cichona plant knew how to treat it

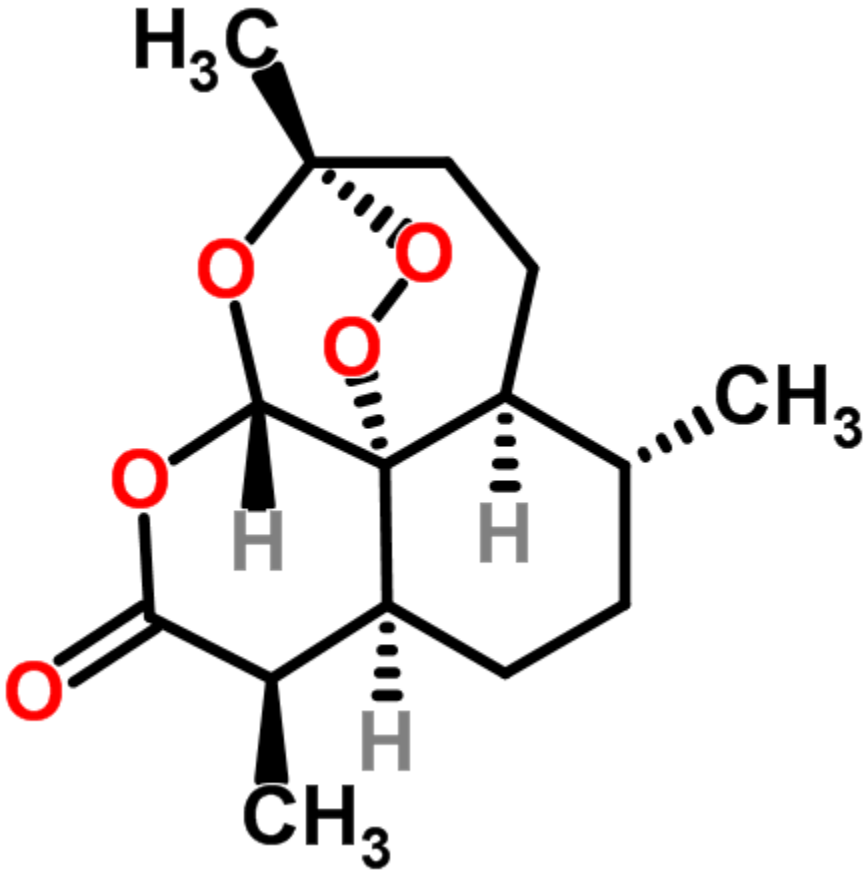


Quinine

After Cinchona, *Artemisia annua* emerged as another plant that knew how to treat malaria



Artemisia annua



Artemisinin

With so many selfless organisms

providing us with great antimalarial medicines,

how does Malaria continue to play havoc

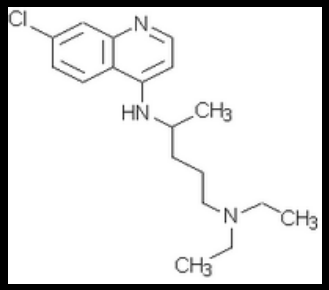
causing economic and emotional turmoil?



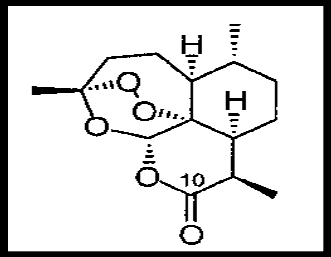
Reason: Drugs Against Malaria come,stay for a while and go



Halofantrine







The euphoria Of CQ

CQ Resistance

Demise of GMEP

GMEP

Alarm! ACTs are on the same path to obsolescence as CQ

Late 1950s

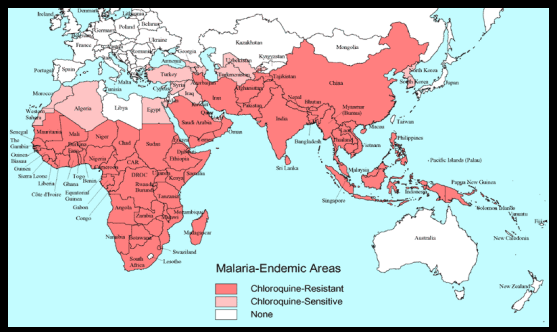
Late 1960s

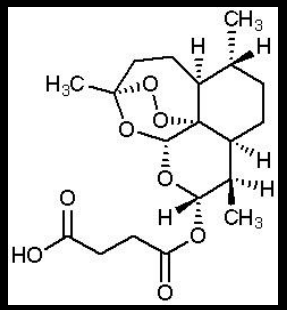
1970s

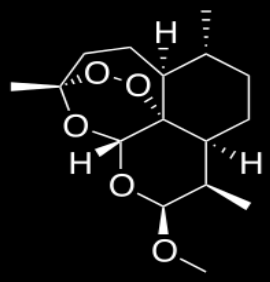
Poor PK but very potent & quite safe.

2005

Resistance to ACTs

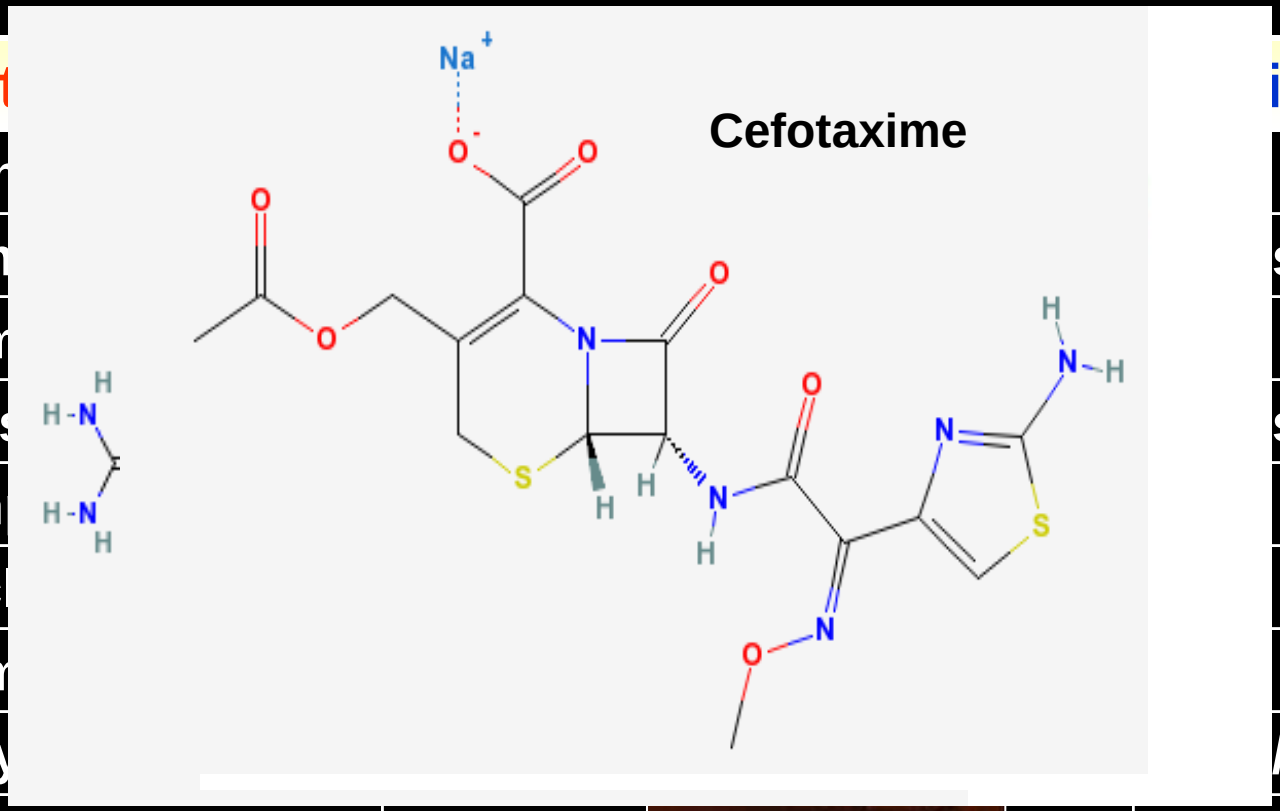






Improved bioavailability+ more effective When combined with Mefloquine (ACT)

The Paradox of drugs is equally true of antibiotics as well
Every time we use an antibiotic we potentially weaken its effectiveness.



Antibiot		istance
Penicillin		
Sulfonam		s
Streptom		
Cephalos		s
Chloram		
Tetracycl		
Erythrom		
Vancomy		/1993
Methicillin	1960	1961
Ampicillin	1961	1973
Cefotaxime/ceftazidime	1981/1985	1988



Moral of the story: Resistance is like a shadow of drugs



If resistance is like a shadow of drugs, the need for new drugs becomes a perennial priority

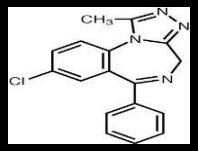
Sahal's laboratory

Consulting Nature for New Drugs Against Malaria

In vivo efficacy



Structure & Synthesis

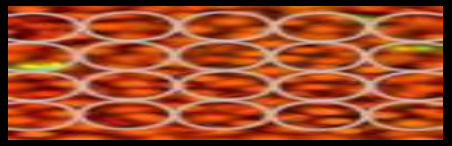


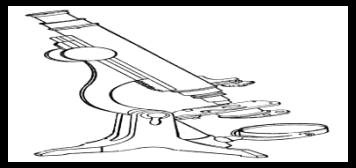
Resistance & Selectivity Indices

Activity guided isolation



Screening







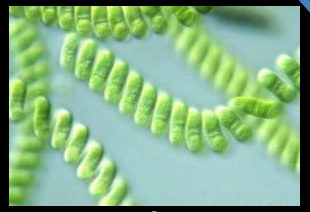
Marine organisms



Medicinal Plants

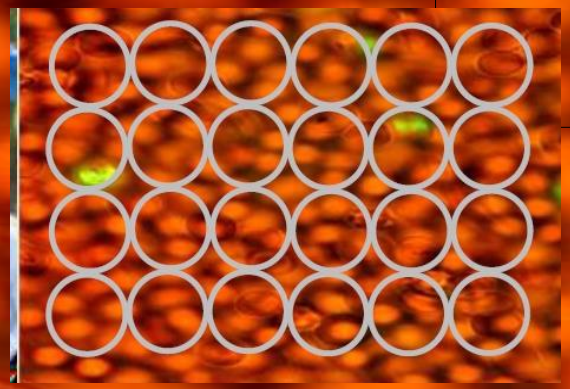


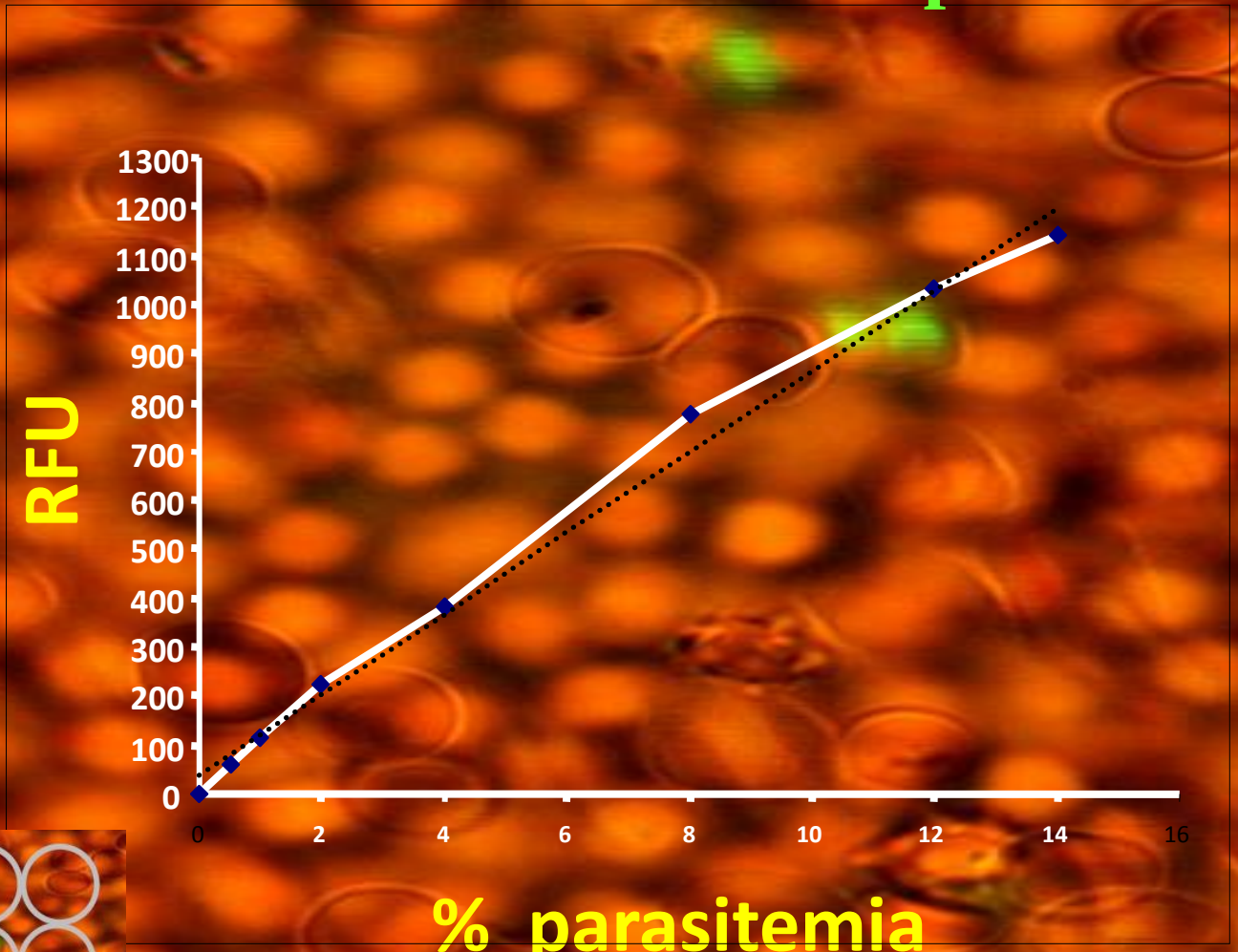
Fungi



Cyanobacteria

Sybr Green fluorescence is α malarial parasitemia



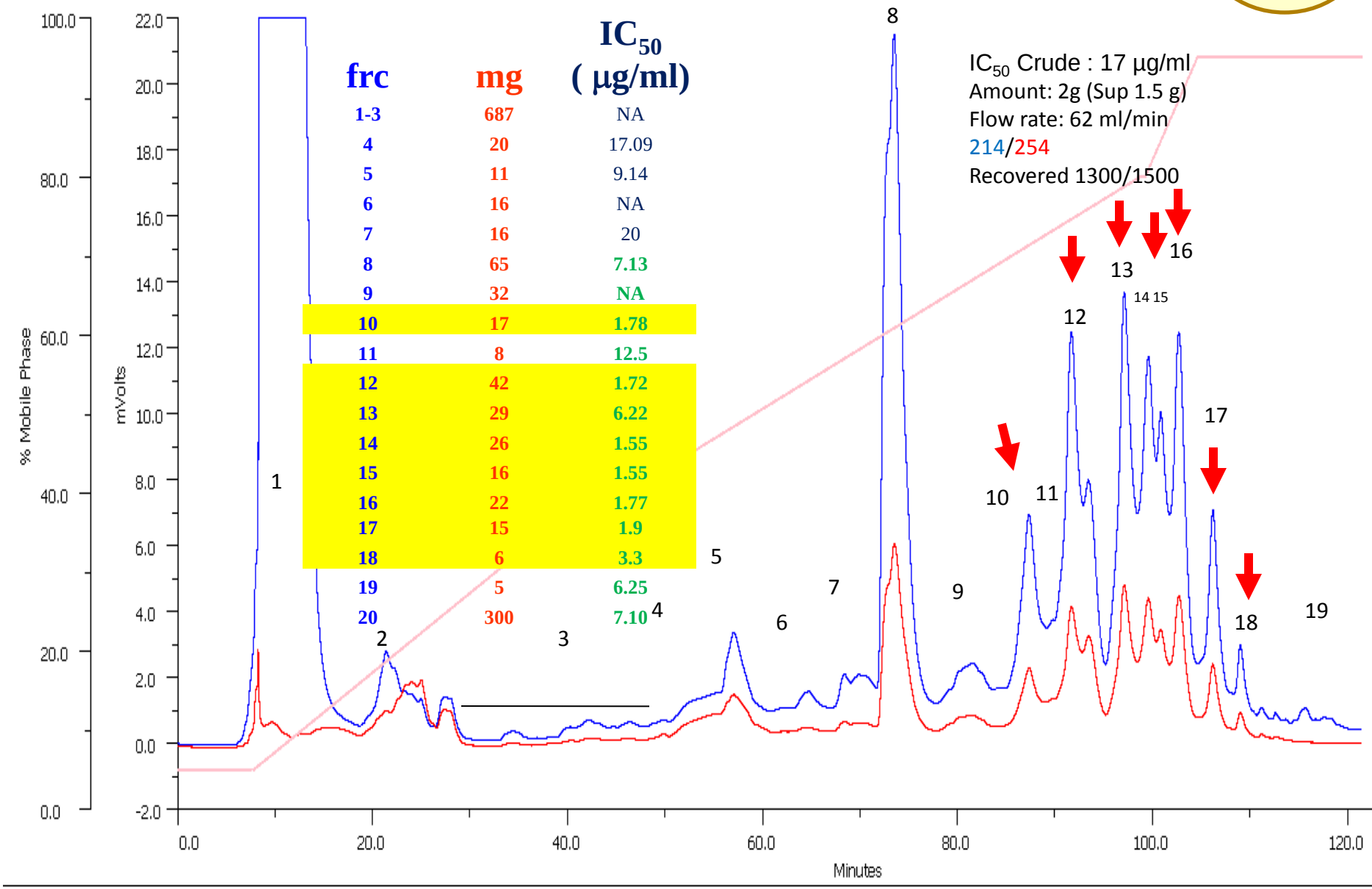


movie

IC_{50} Values of Marine Extracts Showing Antiplasmodial Activity (SYBR Green Method)

Crude Marine Extract	IC $_{50}$ (µg/ml)	Crude Marine Extract	IC $_{50}$ (µg/ml)	Crude Marine Extract	IC $_{50}$ (µg/ml)
2	**10** ***	72	60	**169**	**5** ***
3	Hemolysis	99	15 **	170	22 *
6	50	104	45	171	29 *
11	40 *	106	33 *	***172***	8 ***
13	90	107	80	173	33 *
18	65	109	72	174	60
21	100	***111***	**5** ***	175	39 *
22	80	112	66	176	25 *
40	Hemolysis	114	30 *	**178**	**17** **
54	34 *	115	12 **	179	28 *
55	34 *	119	100	182	26 *
56	90	125	100	184	100
57	30 *	134	100	185	100
58	90	139	41 *	189	100
59	75	142	95	195	37 *
60	100	145	30 *	199	57 *
62	37.5 *	159	32 *	200	29 *
63	68	160	41 *	209	45
64	68	163	32 *	***210***	5 ***
65	36 *	***165***	*8* ***	***217***	<1 ***
66	68	***166***	6.5 ***	***218***	10 ***
67	86	167	89		
71	30 *	168	22 *		

Antiplasmodial activity guided Reverse Phase HPLC separation



frc	mg	IC_{50} (µg/ml)
1-3	687	NA
4	20	17.09
5	11	9.14
6	16	NA
7	16	20
8	65	7.13
9	32	NA
10	17	1.78
11	8	12.5
12	42	1.72
13	29	6.22
14	26	1.55
15	16	1.55
16	22	1.77
17	15	1.9
18	6	3.3
19	5	6.25
20	300	7.10

169 & 178 have a rich spectrum of potent antiplasmodials that are lethal against Chloroquine resistant *Plasmodium falciparum*



169.10 0.13
169.18 &19 0.21
169.14 0.7
169.20 0.8
169.11 1.2
169.17 1.1
169.13 2.6
169.15 2.7
169.16 2.9
169.12 4
169.8 & 22 5
169.21 4.8
169.23 10.5

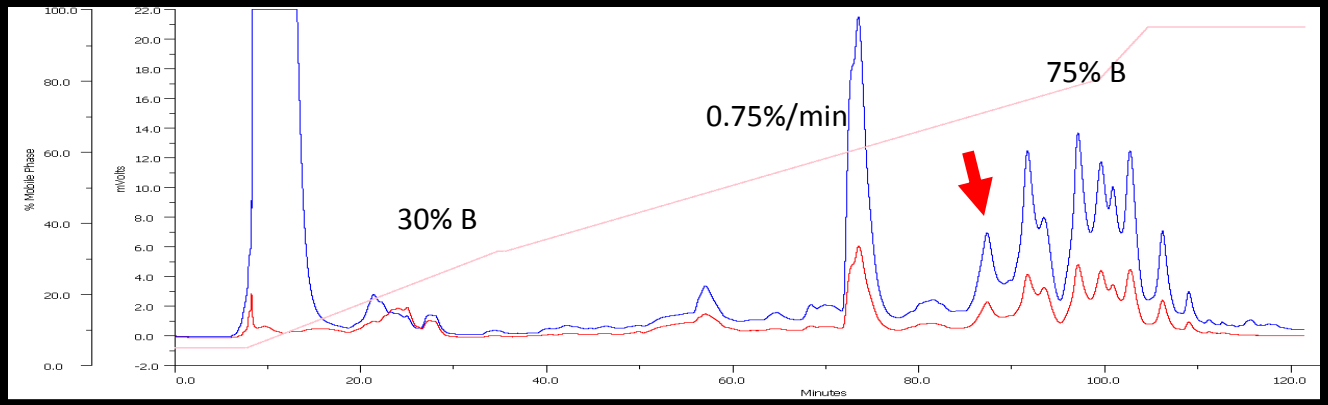
√ 178.17 0.39
178.10 0.39 √
178.15 1.5
178.14 1.5
178.16 1.8
178.12 1.7
178.18 3.3
√ 178.13 6.22
178.19 6.25
178.8 7.13
178.5 9.14
178.11 12.5

Fraction no → 169.10 ← Extract no
IC$_{50}$ (μg/ml) → 0.13



√: potent against Chloroquine resistant *Pf* RKL9

Antiplasmodial activity guided purification of 178.10



IC_{50}
17 µg/ml

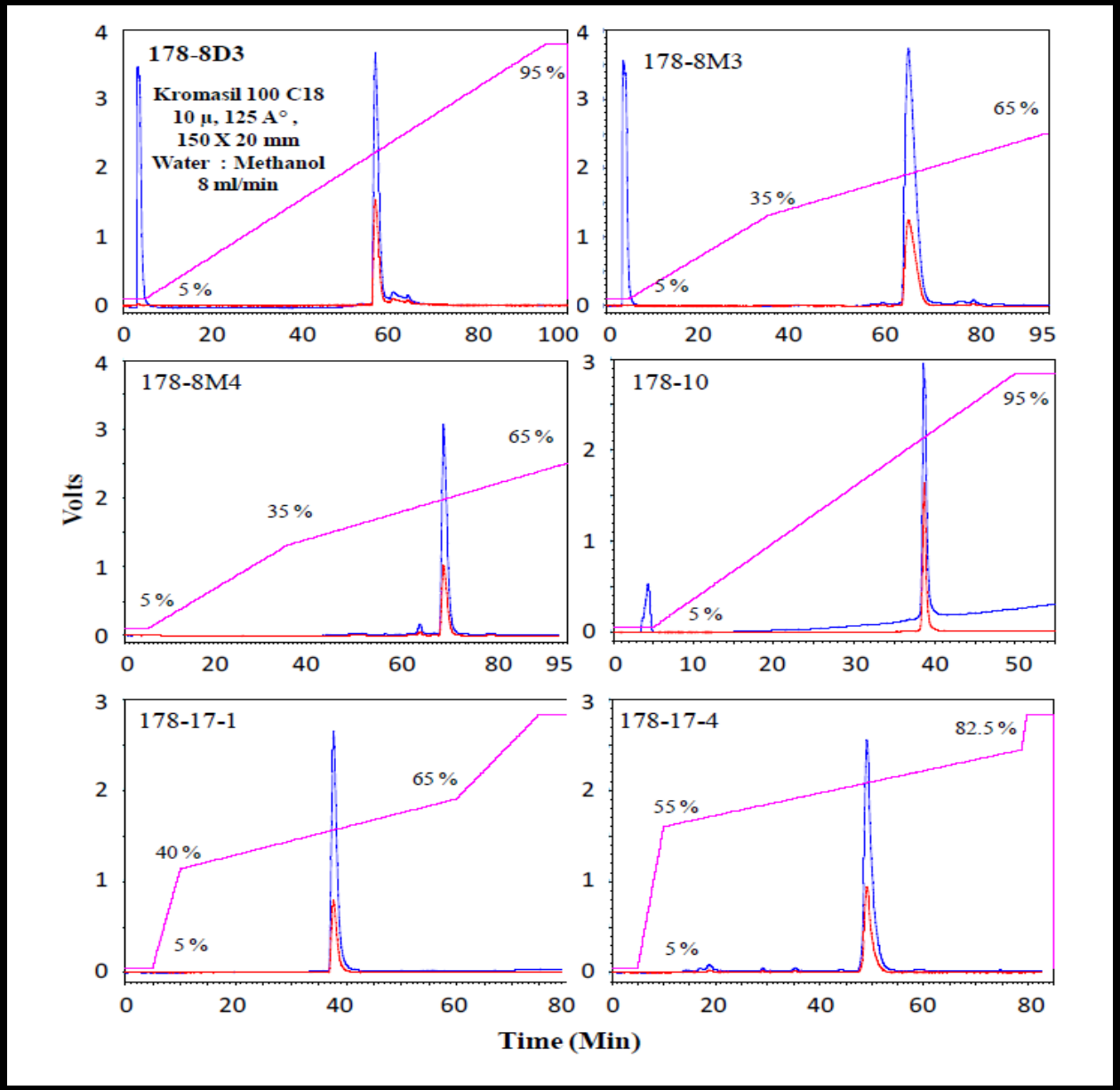
2 g

62 ml/min

15 µm

Antiplasmodial molecules purified from 178





Antiplasmodial potencies, mammalian cell cytotoxicities and selectivity indices of ME178 derived molecules.

Sr. No	Sample ID	*P. falciparum* IC_{50} (μg/ml)
		3D7
1	178D3	1.2
2	178M3	0.7
3	178M4	0.9
4	178-10	0.39
5	178-13-1	0.54
6	178-13-2	0.6
7	178-17-1	0.42
8	178-17-4	0.84
9	178-17-6	0.78

Nature inspired chemical synthesis of new pharmacophores Against Malaria

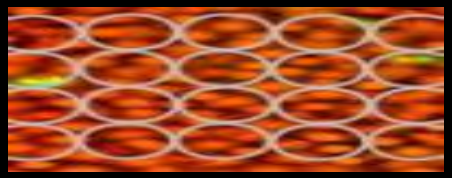


In vivo efficacy

PK



Resistance & Selectivity Indices





Screening

Nature inspired chemical libraries based on Chalcones, Stilbenes Dibenzylideneacetones, Acridines, Quinolines, Pyrimidines, Cryptolepins, Azoles, Aplysinopsins, Chromones, Bisindoles, Indoquinolines , Phloroglucinols Dendrimeric peptides & metallic nanoparticles

Nature inspired chemical synthesis of new pharmacophores Against Malaria

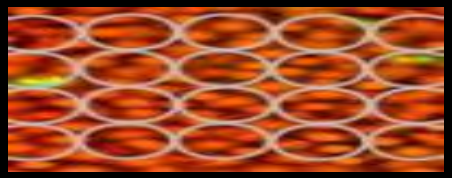


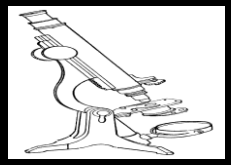
In vivo efficacy

PK



Resistance & Selectivity Indices





Screening

Nature inspired chemical libraries based on

Chalcones, Stilbenes Dibenzylideneacetones,

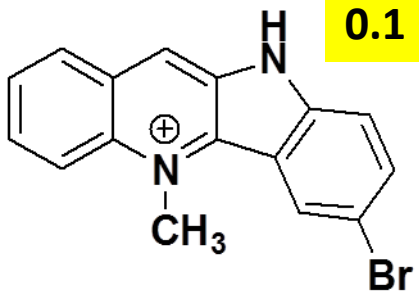
Acridines, Quinolines, Pyrimidines, Cryptolepins,

Azoles, Aplysinopsins, Chromones, Bisindoles, Indoquinolines

, Phloroglucinols Dendrimeric peptides metallic nanoparticles

A glimpse of chemically synthesized molecules active against malaria parasite

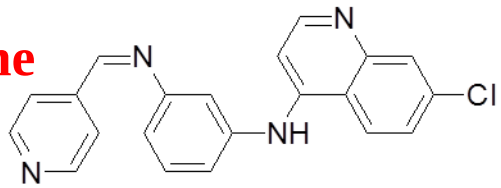
Cryptolepine
IIIM-MCD-668



0.1 150

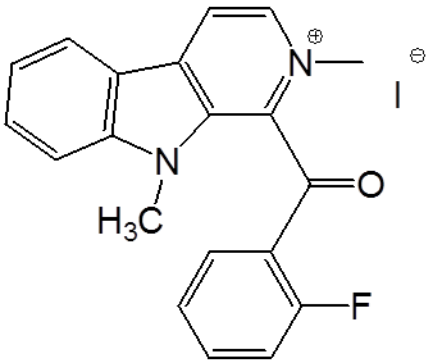
0.34 217

Aminoquinoline
S5



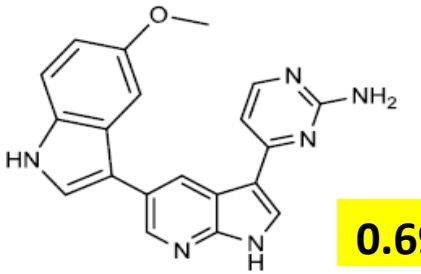
0.47 213

Bisindole
IIIM-MCD-422





Pyrimidine
IIIM/724/ 1582/CF/08
IC_{50} : 0.69 µM/ --

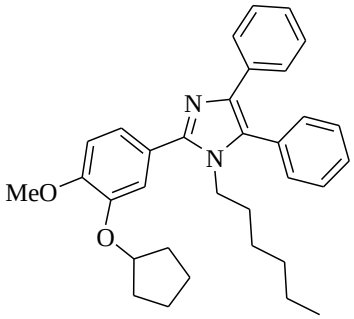


0.69 ---

Pf 3D7IC_{50} (µM) Selectivity index

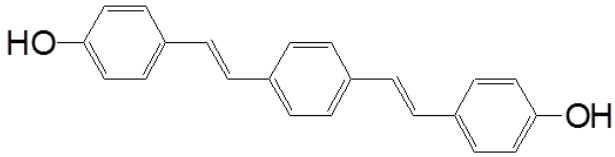
0.75 >133

Imidazole
GVM-GV-010



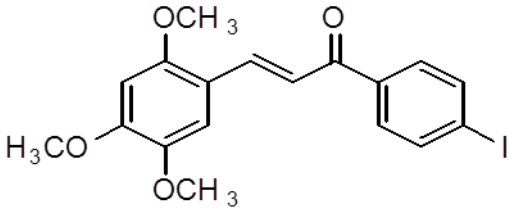
0.9 >111

Stilbene
i10-183



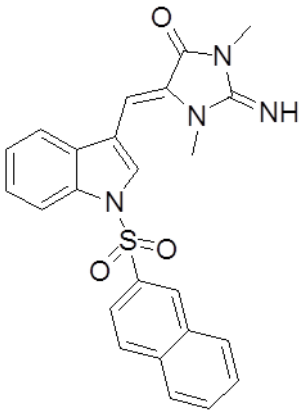
2 50

Chalcone
i9-36



2.8 >36

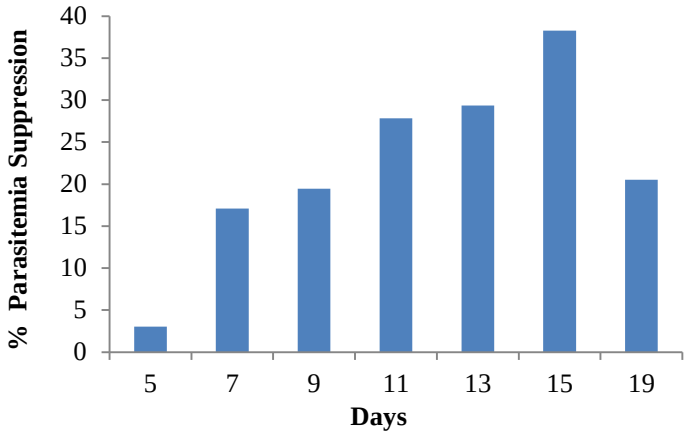
Aplysynopsin
IIIM-MCD-680

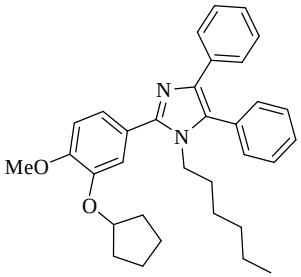


In vivo antimalarial activity of orally administered GV10 in *P. berghi* (ANKA) infected *Balb/C* Mice

Vehicle: 2% (Hydroxypropyl) methyl cellulose with 2 % Tween 80 in Normal saline
Dose : 50 mg/KgBW in 200 µL /mouse
Route : Oral
Mice/ Group: 5 ♀
IC_{50} (µM) *Pf*3D7: 0.75 µM ; *Pf* INDO: 0.8 µM ; **Selectivity Index: >133.3 (HeLa)**







% Parasitemia

90 80 70 60 50 40 30 20 10 0

GV10

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Days

IV injection of Infected RBC (10^5)

Untreated control

GV10

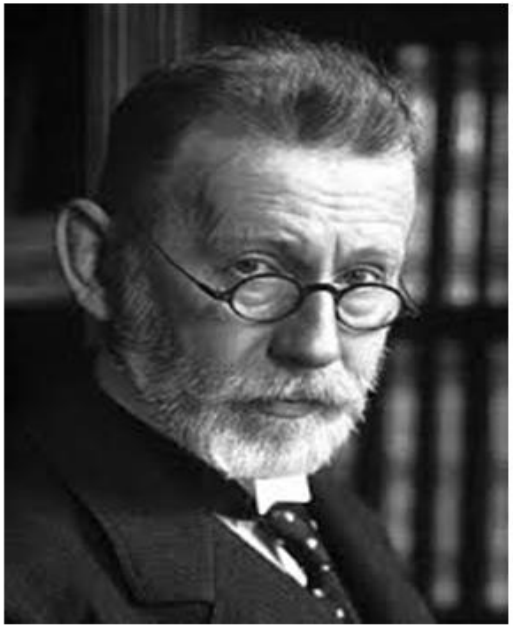
Average survival time (Days)	
Untreated Control	**GV10**
20.2 ± 2.3	**28.6 ± 4.2**

With both Nature and the ingenious chemists

on our side

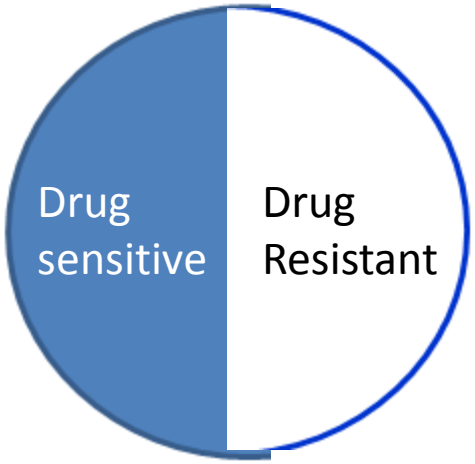
Why have we allowed Malaria to

continue to rattle us?



The 19th century concept of “magic bullets” paid an important role in standardization of pharmaceuticals. Drugs would work perfectly as long as the pathogen was “ the same”.

But such “single magic bullet” would stop working when the pathogen learnt to survive with mutations.

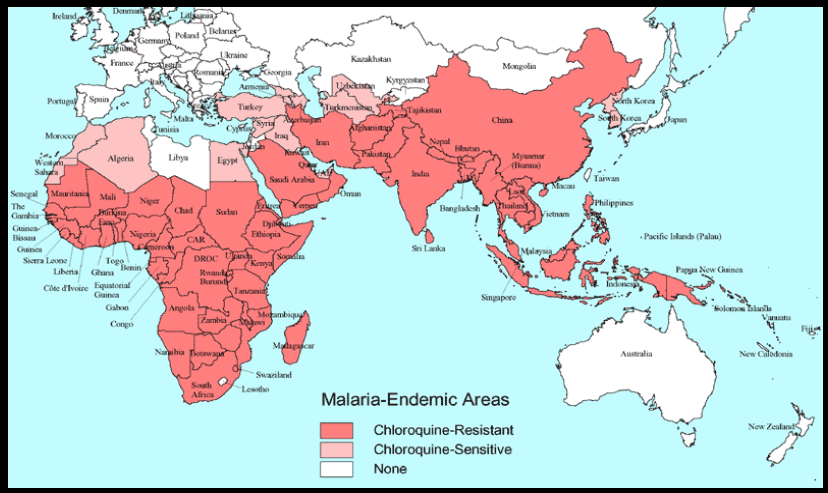


The two halves of the 19th century

Benefits of 1st half were undone in the 2nd half of 19th century by proliferation of resistance to antibiotics, Antimalarials, Insecticides & herbicides.

As long as crude extracts of medicinal plants were in use, we had no problem of resistance

Resistance became common place since the time we started using modern medicine which professed the use purified Single molecule based drugs which worked like magic bullets



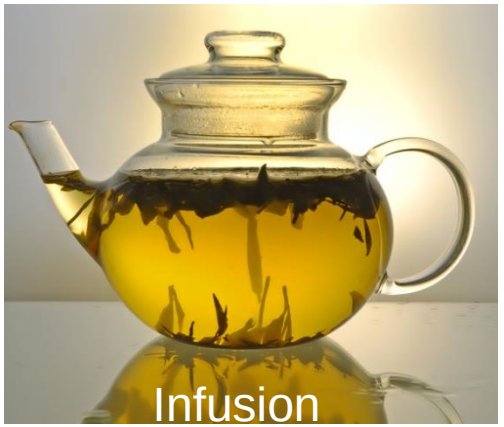
Has rational scientific thinking that pulled drugs out from plants been responsible for drug resistance?

WHO's warning is valid because of low Artemisinin content of :


Juice extraction



Tea


Infusion

But what about oral consumption of dried leaves of the whole plant?



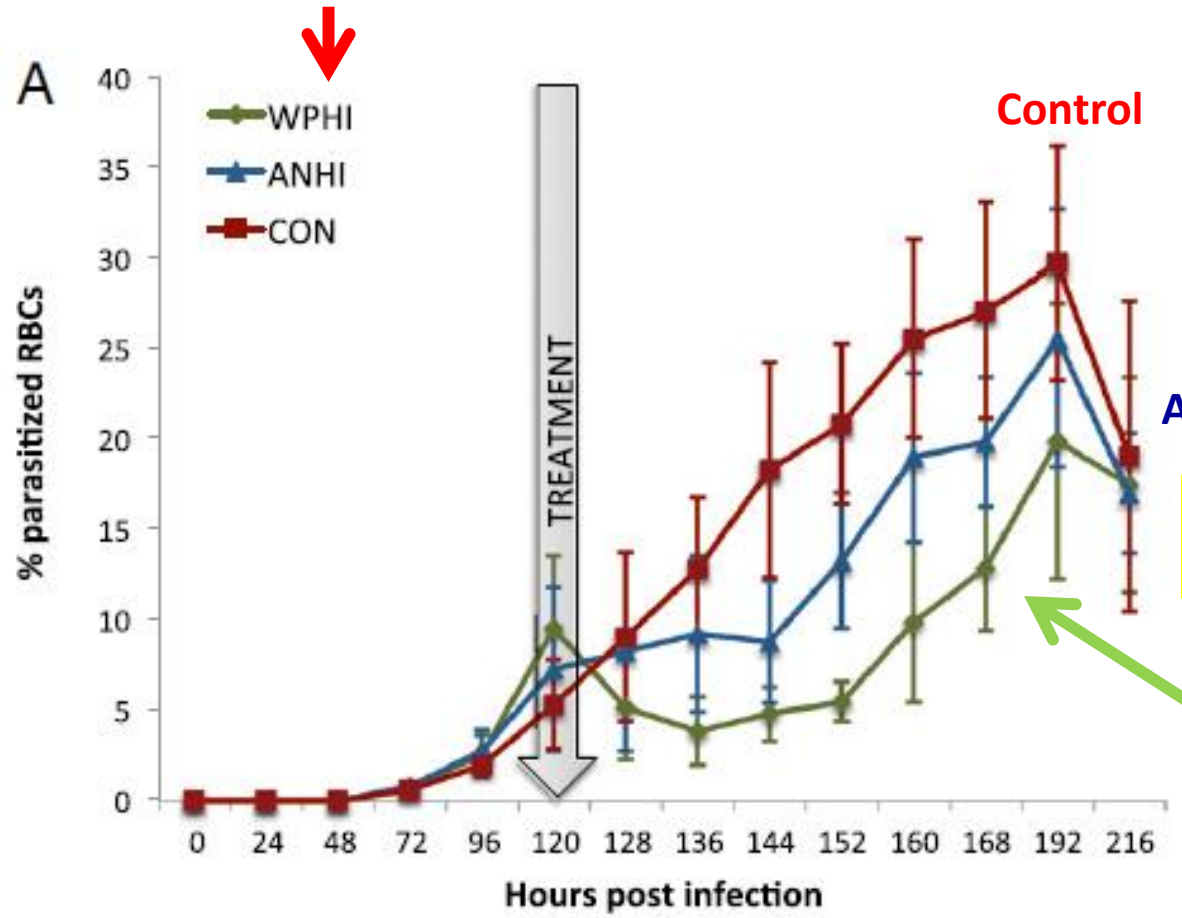
Dried whole-plant *Artemisia annua* slows evolution of malaria drug resistance and overcomes resistance to artemisinin

PNAS 2015

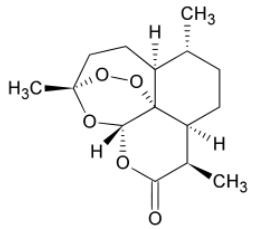
Mostafa A. Elfawal[a], Melissa J. Towler[b], Nicholas G. Reich[c], Pamela J. Weathers[b], and Stephen M. Rich[a,1]



Whole-plant *Artemisia annua* restricts the growth Artemisinin resistant P.yeolii (ART) much better than pure Artemisinin each at the high dose of 120 mg/Kg Artemisinin



Control

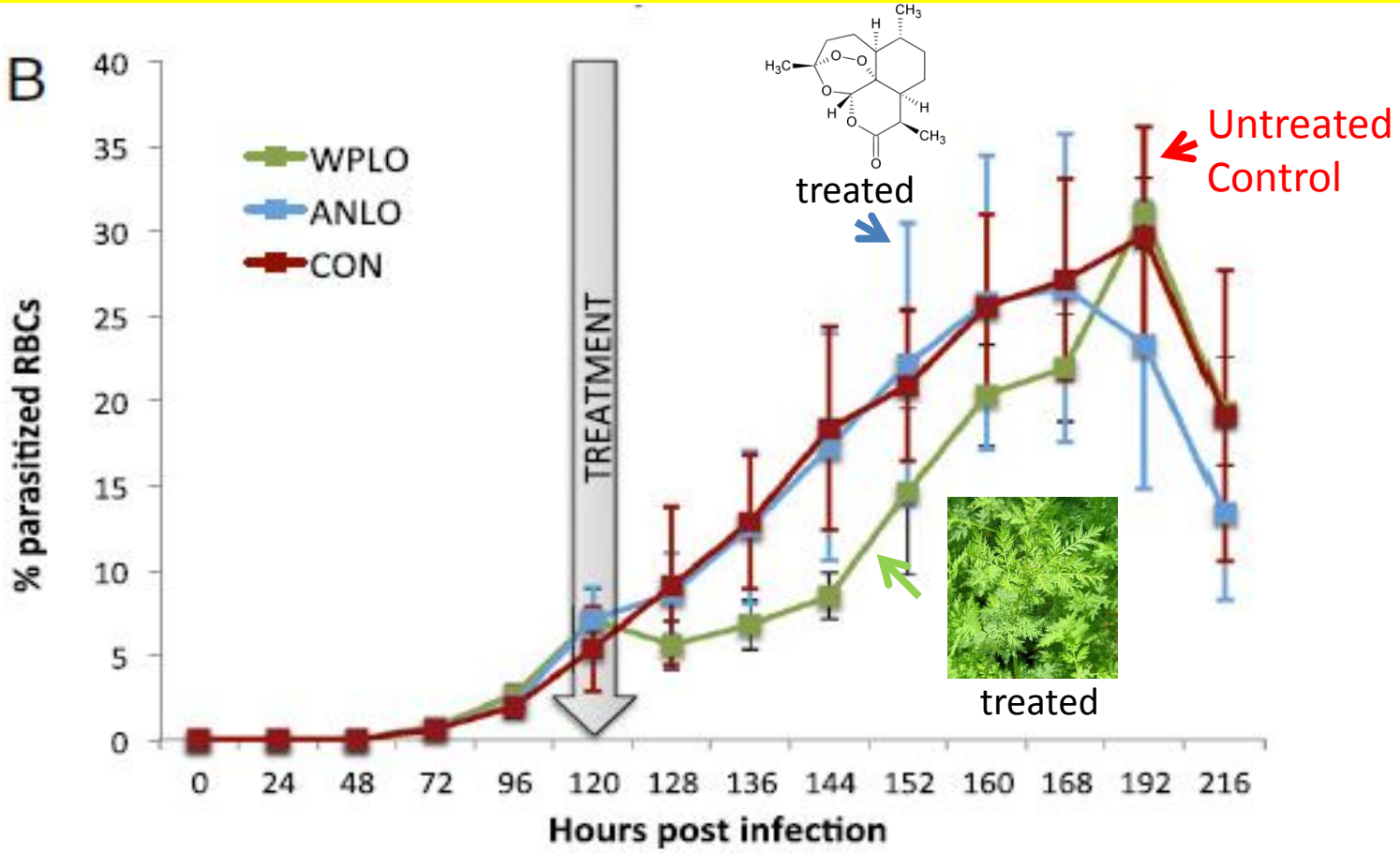


Artemisinin (AN) treated

A.Annua whole plant (WP) treated

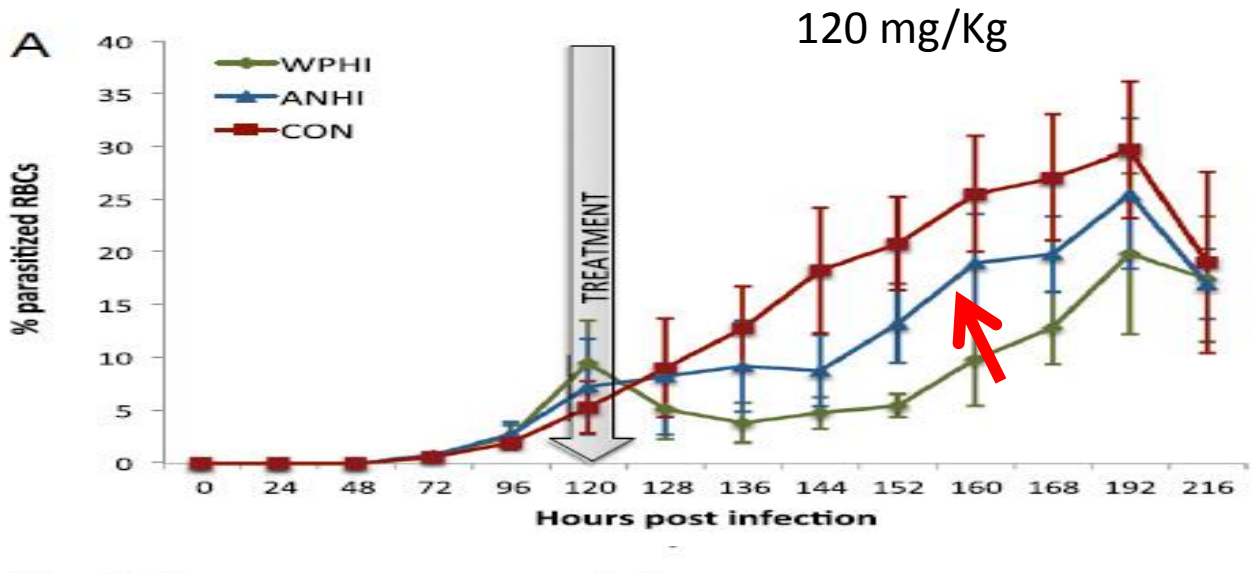


At the lower dose of 24 mg/Kg Artemisinin, pure Artemisinin is ineffective while the whole plant shows growth retardation of Artemisinin resistant P.yeolii (ART)



$AN^{HI}= WP^{LO}$

HI

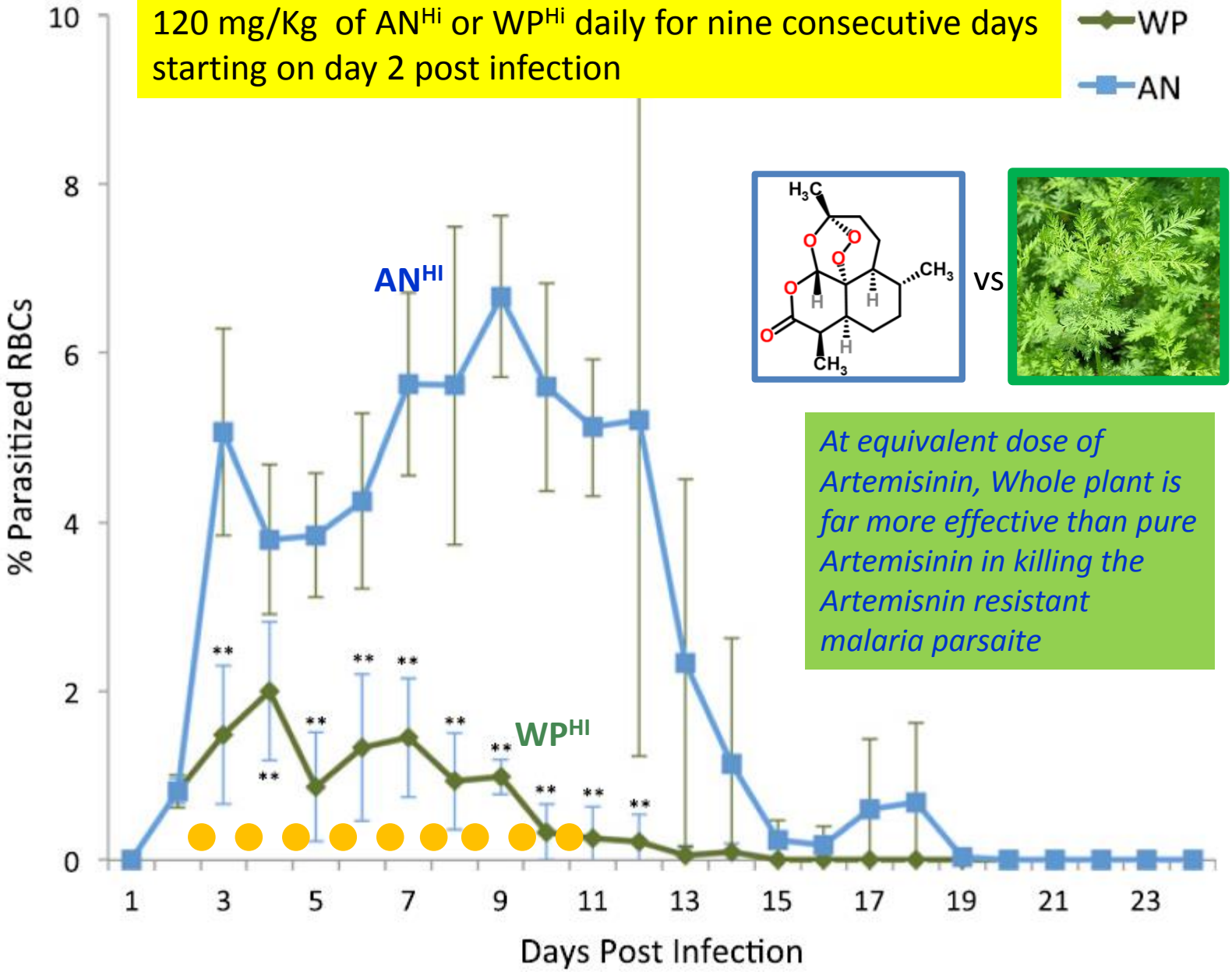


120 AN/24WP=5.

The plant is 5 times more effective than purified Artemisinin in restricting the growth of Artemisinin resistant *P.yeolii* (ART) Malaria parasite.

AN-resistant *P.yoelii* curative treatment

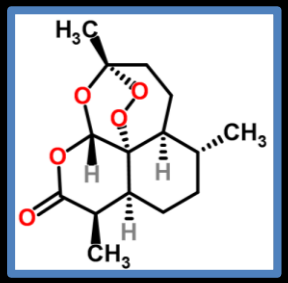
120 mg/Kg of AN^{Hi} or WP^{Hi} daily for nine consecutive days starting on day 2 post infection



At equivalent dose of Artemisinin, Whole plant is far more effective than pure Artemisinin in killing the Artemisnin resistant malaria parsaite



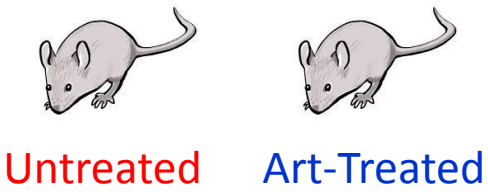
Could nonpharmaceutical whole plant forms of therapy abolish the shadow of resistance associated with monotherapies?



Artificial Evolution Experiment to compare rates at which resistance arises against Artemisinin VS *Artemisnin annua*

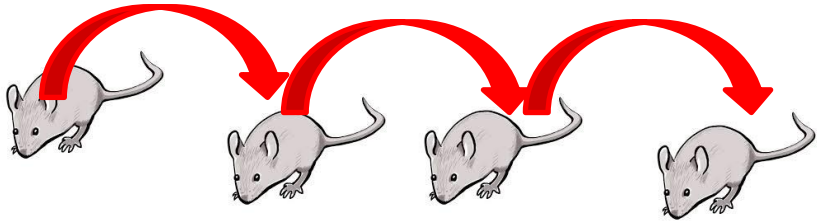
2% DT: Difference in time to reach 2% parasitemia between treated and untreated animals

P chabadui (Artemisinin sensitive) infected mice

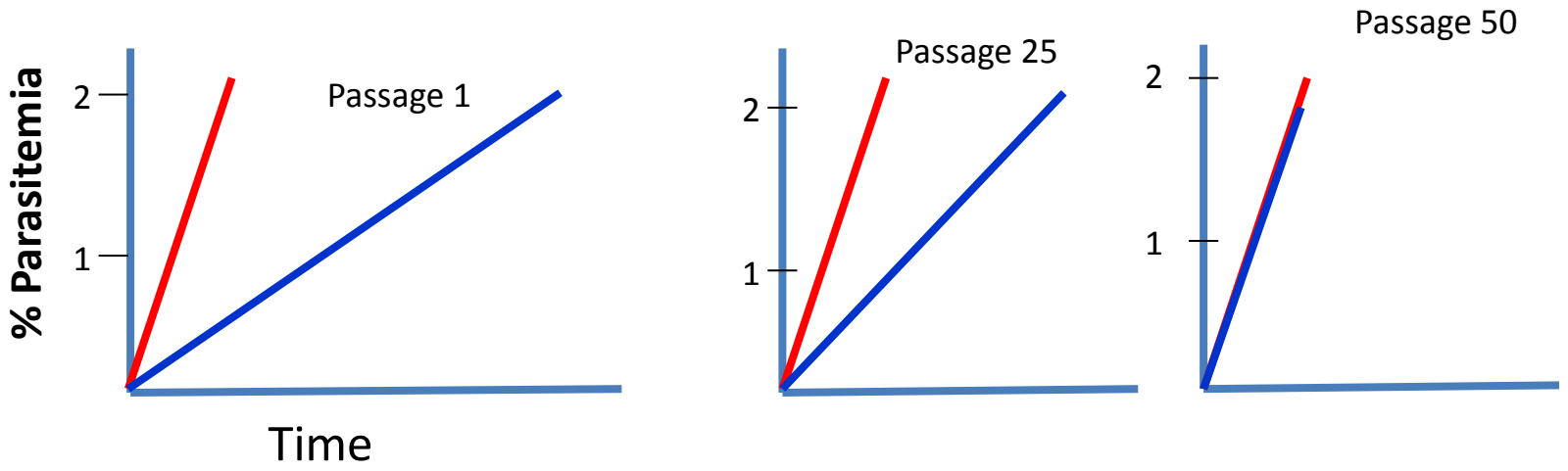


Untreated Art-Treated

Passaging blood from mouse to mouse



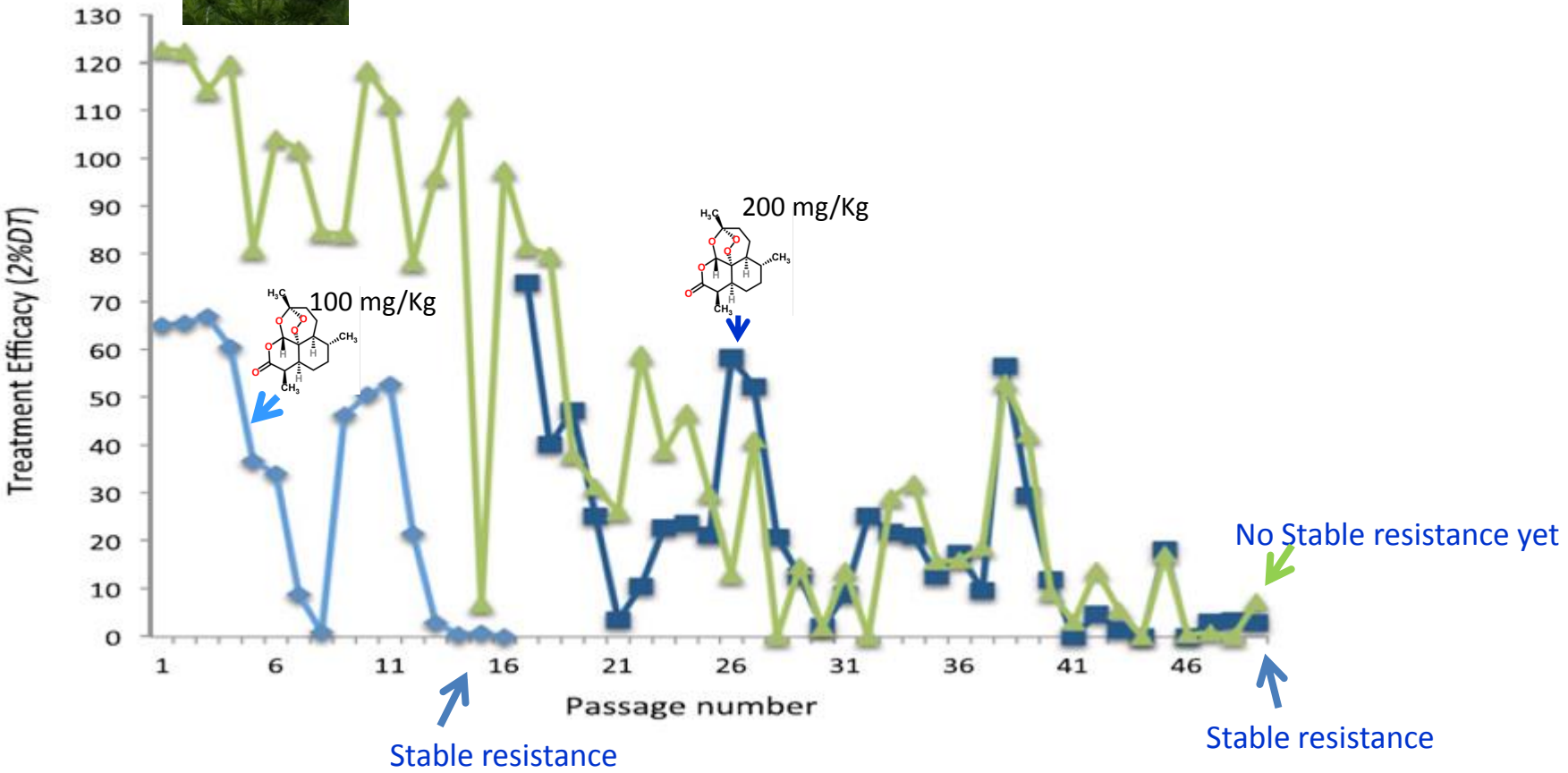
Artemisinin treated



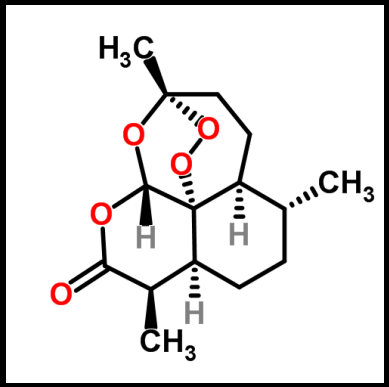
Whole plant treatment of 100 mg/Kg is more resilient than 200 mg/Kg of Pure Artemisinin in resisting the emergence of resistance

2%DT





What may cause the whole
Plant to be more potent
& reliable
than the plant derived
magic bullet alone?

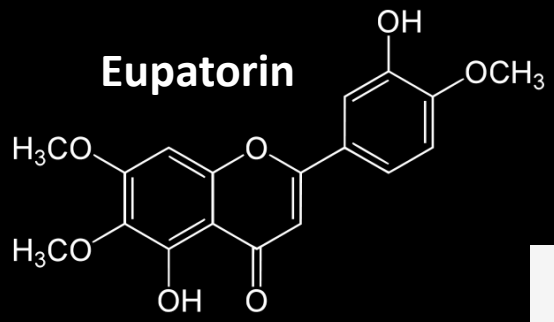




Artemisia annua

Contributory factors: (i) A.annua flavinoids with Antimalarial activity

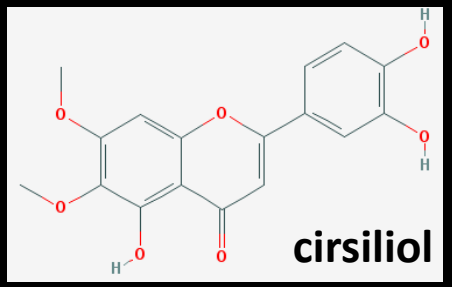
Eupatorin



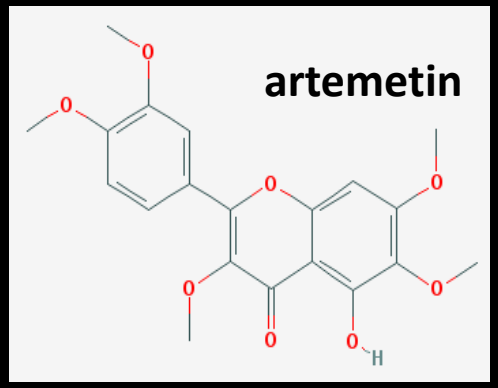
Kaempferol





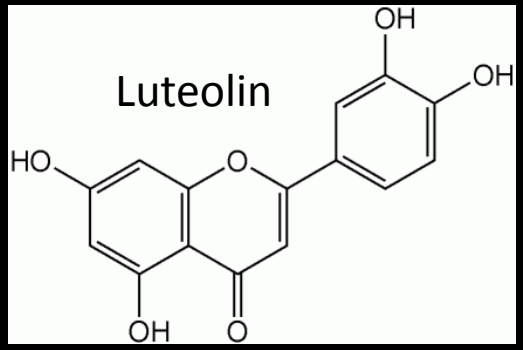


cirsiliol



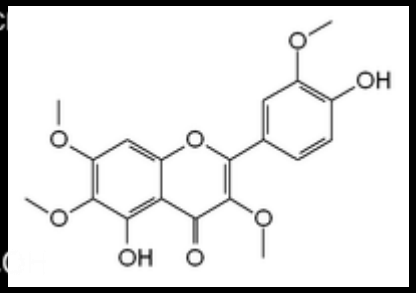
artemetin

Luteolin

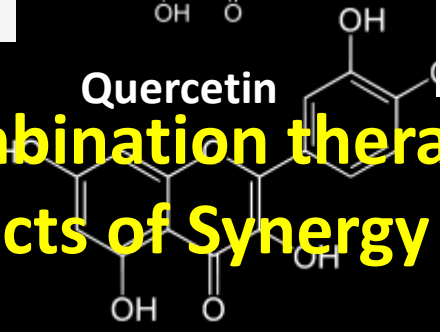


Casticin



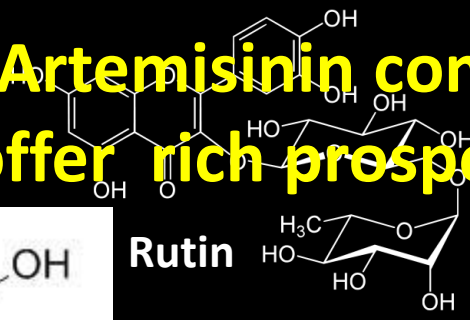


Quercetin

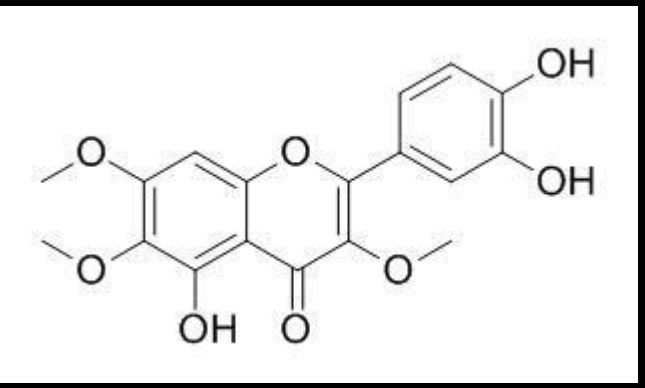


Chrysosplenetin

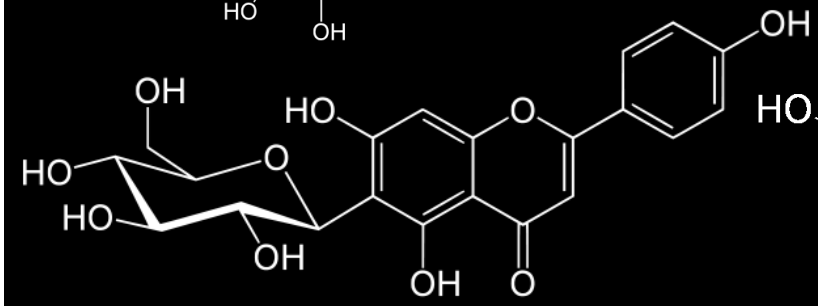
Plant based Artemisinin combination therapy (pAct) can offer rich prospects of Synergy



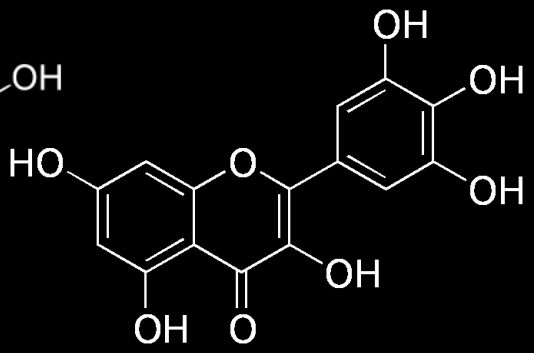
Rutin



Chrysosplenol D

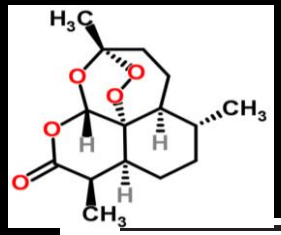


Isovitexin



Myricetin

(ii) Whole Plant enhances bioavailablity of its "magic bullet"



: In serum 60 min post pACT or pure Artemisinin gavage

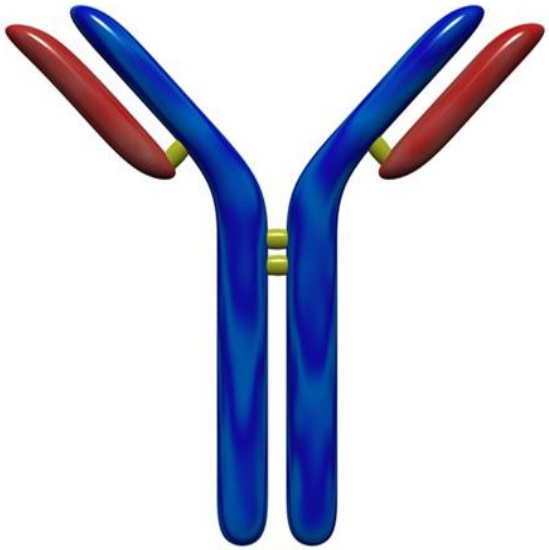
Mouse treatment	Artemisinin (mg L^{-1})
Healthy untreated (3 pooled mice)[a]	0
Healthy + 100 mg AN kg^{-1} (3 pooled mice)[a]	0

[a] These mice were treated in groups of three; blood was harvested and pooled prior to extraction and measurement of artemisinin, so SE was not calculated.

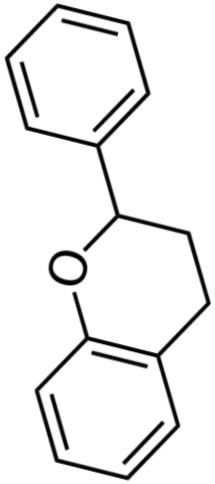
[b] Values are ± SE.

Chou contains soy, oats, wheat,beet pulp, corn etc

(iii) Animals and Plants use interestingly different biological warfar strategies to fight similar pathogens

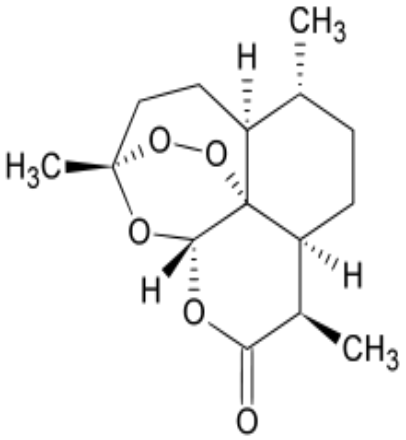


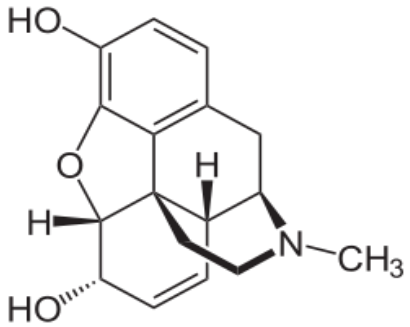




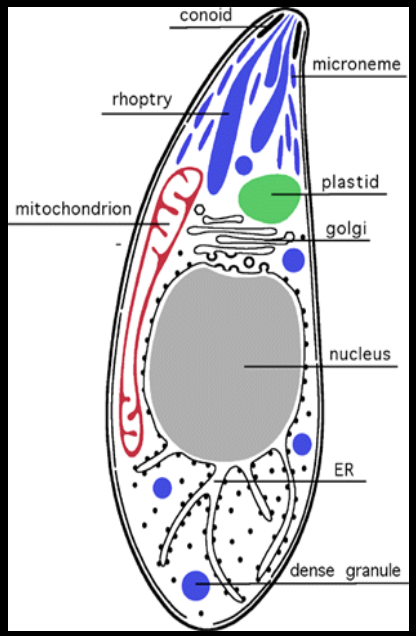


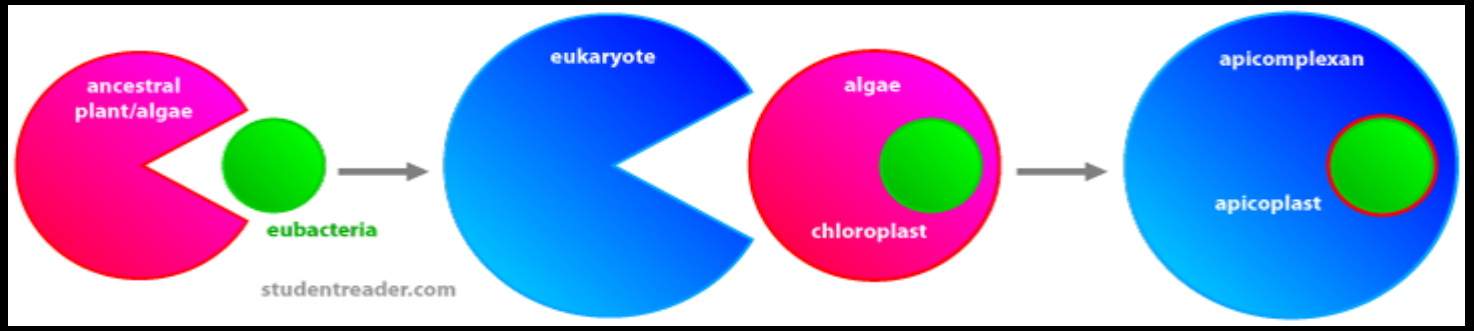






(iv)That these defences are also effective at killing a mammalian blood borne protozoan parasite seems coincidental; but there is a plausible evolutionary explanation for this cross- species protection.





In retrospect, In matters of biological warfare and drugs, Plants are more full of wisdom than us humans



Because if Plants had followed the pharmaceutical model of serial production of single-component protection against its enemy, they would have become extinct long ago.

Our century needs a sense of direction in the use of drugs

Pharmacutical based monotherapeutics	Plant based polytherapeutics
Predetermined content, fully standardized	Variable content, lack of standardization
Lower potency	Greater potency
Resistance inevitable	Resistance is less frequent
Consistent with a fast life style	Work well where life is in harmony with Nature
Generally safe	Friendly and toxic molecules may coexist

How can we combine the virtues of single component therapeutics with the virtues of the multicomponent therapeutics ?

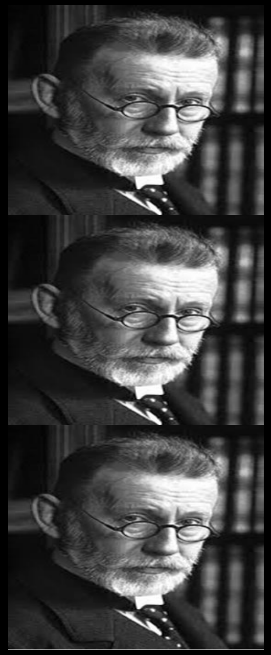
Answer: By identifying the virtues of each molecule in a repertoire of pure magic bullets, & mixing them all before making a composite pill for the patient.



Time

Magic Bullet

Resistance



Synergy

Enhancer of bioavailability

Greater Potency
&
Prolonged efficacy