

REVIEWS

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# Analysis of additivity and synergism in the anti-plasmodial effect of purified compounds from plant extracts

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## Abstract

In the search for antimalarials from ethnobotanical origin, plant extracts are chemically fractionated and biological tests guide the isolation of pure active compounds. To establish the responsibility of isolated active compound(s) to the whole antiplasmodial activity of a crude extract, the literature in this field was scanned and results were analysed quantitatively to find the contribution of the pure compound to the activity of the whole extract. It was found that, generally, the activity of isolated molecules could not account on their own for the activity of the crude extract. It is suggested that future research should take into account the “drugs beside the drug”, looking for those products (otherwise discarded along the fractionation process) able to boost the activity of isolated active compounds.

## Introduction

In the search for anti-malarial activity of plants traditionally used against fevers, collected plants are first submitted to an extraction process with polar or apolar solvents. Ideally, the extracts are then tested against erythrocytic stages of *Plasmodium falciparum in vitro* to validate anti-plasmodial activity. Classically, when biological tests identify significant activity, crude extracts are submitted to a bioguided fractionation procedure, aiming to isolate the active compound(s). For that purpose, several sequential extractions with solvents of diverse polarities are performed, and purified fractions are submitted to anti-plasmodial tests and to chemical identification. Frequently, many promising extracts are discarded because the anti-plasmodial activity disappears along the fractionation process. The failure to isolate active constituents from active extracts may be due to the lability/instability of the active compounds that are degraded during the extraction process. Sometimes, the loss of activity is due to the fact that the compounds display their activity only when they interact in the crude extract. Such compounds will be lost for further

development unless their interactions can be examined. In order to evaluate such interactions, mostly synergistic, it is necessary to know the inhibitory activity of the crude extract and the purified fractions (i.e., their IC<sub>50</sub> values) and the yields of extraction of the purified compounds to allow calculation of their absolute quantitative prevalence in the extract. Unfortunately, in most cases, when plants are extracted and fractionated, the activity of the crude extract is not determined and the yields are not reported or not determined altogether. This is the case of hundreds of thousands of purified fractions of natural extracts that have been evaluated by cell-based inhibition tests.

To determine the quantitative contribution of the pure compounds to the activity of a crude extract, data from the literature were compiled selecting those publications in which the activities of the crude extracts and of the purified compounds (and their yields) were reported. To calculate the contribution of the pure active compound to the activity of the extract, the respective IC<sub>50</sub> values and the yield of the purified compound are used. Data are shown in table 1.

The equation describing the relationship between concentration and IC<sub>50</sub> is:

$f = \frac{\max - (\max - \min)}{1 + x/EC_{50}}^{\text{slope}}$  where “f” is the inhibitory effect. The EC<sub>50</sub> is the IC<sub>50</sub> of the isolated compound and “x” is the yield-dependent calculated concentration of

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**Table 1 Compilation of data from the literature on the anti-plasmodial effects of plant extracts and their fractionated active compounds. CS and CR are chloroquine-sensitive and -resistant strains respectively**

Plant Species	Family	Parasite strain	Extract IC <sub>50</sub> µg/ml	Most active compounds*	Compounds IC <sub>50</sub> µg/ml	Yield %	% of active comp of extract IC <sub>50</sub>	Contribution of active compound to extract inhib %	ref #
<i>Alstonia macrophylla</i> Wall.	Apocynaceae	Pf K1 CR	5,7	Macrocarpamine	0,27	0,95	0,05	33,4	1
<i>Alstonia macrophylla</i> Wall.	Apocynaceae	Pf K1 CR	5,7	Villalstonine	0,17	0,6	0,03	31,1	2
<i>Artemisia indica</i> Willd	Asteraceae	Pf K1 CR	6,6	Exigua flavanones	4,6	0,15	0,01	0,4	3
<i>Brucea javanica</i> L. (Merr.)	Simaroubaceae	Pf K1 CR	0,5	Brucein	0,005	0,002	0,00001	0,4	4
<i>Cryptolepis sanguinolenta</i> (Lindl.)	Apocynaceae	Pf K1 CR	5,41	Cryptolepine	0,054	0,04	0,002	7,7	5
<i>Diospyros sanzaminika</i> A. Chevalier	Ebenaceae	Pf K1 CR	0,8	4-O-(3'-methylgalloyl) norbergenin	0,6	1,2	0,01	3,1	6
<i>Erythrina fusca</i> Lour.	Fabaceae	Pf K1 CR	7,5	Citflavanone	5	0,1	0,01	0,4	7
<i>Erythrina fusca</i> Lour.	Fabaceae	Pf K1 CR	7,5	Lonchocarpol	1,6	0,2	0,02	1,9	7
<i>Erythrina fusca</i> Lour.	Fabaceae	Pf K1 CR	7,5	8-Prenylidaidzein	3,9	0,0006	0,00005	0,002	7
<i>Garcinia cowa</i> L.	Clusiaceae	Pf T9/94CS	5	7-O-Methylgarcinone	2,5	0,01	0,00030	0,02	8
<i>Garcinia cowa</i> L.	Clusiaceae	Pf T9/94CS	5	Cowanin	3	0,2	0,01	0,7	8
<i>Garcinia cowa</i> L.	Clusiaceae	Pf T9/94CS	5	Cowanol	1,6	0,5	0,03	3,1	8
<i>Garcinia cowa</i> L.	Clusiaceae	Pf T9/94CS	5	Vowaxanthone	1,5	0,4	0,02	2,6	8
<i>Garcinia cowa</i> L.	Clusiaceae	Pf T9/94CS	5	b-Mangostin	3	0,04	0,002	0,1	8
<i>Geissospermum sericeum</i> Miers	Apocynaceae	Pf K1 CR	1,78	Flavopereirine	2,84	0,04	0,0008	0,06	9
<i>Gomphostemma niveum</i> Hook. f.	Lamiaceae	Pf MR-C02 CS	9,7	Gomphostenin	38,2	0,5	0,05	0,3	10
<i>Gomphostemma niveum</i> Hook. f.	Lamiaceae	Pf MR-C02 CS	3,4	Gomphostenin-A	3,4	24	0,83	39	10
<i>Guiera senegalensis</i> J. F. Gmel.	Combretaceae	Pf W2 CR	4,45	Harman (b-carboline)	3,29	0,1	0,00445	0,3	11
<i>Holostylis reniformis</i> Duch.	Rubiaceae	Pf BHZ26/86 CR	0,7	Lignan	0,12	0,4	0,003	4,6	12
<i>Holostylis reniformis</i> Duch.	Rubiaceae	Pf BHZ CR	0,7	Lignan	0,12	4,5	0,03	42	12
<i>Nauclea orientalis</i> L.	Rubiaceae	Pf D6 CS	3	Oleanolic acid	4,6	0,07	0,002	0,08	13
<i>Phyllanthus niruri</i> L.	Euphorbiaceae	Pf CS	1,3	Terpenes	1,3	0,1	0,002	0,3	14
<i>Piptadenia pervillei</i> Vatke (Entada pervillei Vatke (R.Vig.)	Fabaceae	Pf MCF29	3,7	Catechin derivatives	0,4	0,03	0,001	0,6	15
<i>Piptadenia pervillei</i> Vatke (Entada pervillei Vatke (R.Vig.)	Fabaceae	Pf FcM29 CR	3,7	Catechin derivatives	0,3	0,1	0,004	2,4	15
<i>Pleiocarpa mutica</i> Benth.	Apocynaceae	Pf K1 CR	16,7	Pleiomutinine	3,2	0,05	0,008	0,5	16
<i>Polyalthia debilis</i> (Piere) Finet & ganep	Annonaceae	Pf K1 CR	1,35	Bis-dehydroaporphine	4,1	0,16	0,002	0,1	17
<i>Pothomorphe peltata</i> L.	Piperaceae	Pf K1 CR	3,7	4-Nerolidylcatechol	0,21	5,7	0,21	100	18
<i>Quassia amara</i> L.	Simaroubaceae	Pf W2 CR	8,9	Simalikalactone D	0,005	0,001	0,0001	3,5	19

**Table 1 Compilation of data from the literature on the anti-plasmodial effects of plant extracts and their fractionated active compounds. CS and CR are chloroquine-sensitive and -resistant strains respectively (Continued)**

<i>Rhaphidophora decursiva</i> Schott	Araceae	Pf W2 CR	6,8	Polysyphorin	0,37	0,00004	0,000003	0,001	20
<i>Rourea minor</i> (Gaertn.) Alston	Connaraceae	Pf W2 CR	2	Rourinoside (glycoside)	1,2	4	0,08	12,5	21
<i>Stephania pierrei</i> Diels	Menispermaceae	Pf W2 CR	3	Asimilobine	0,4	0,3	0,008	3,7	22
<i>Strychnos icaja</i> Baillon	Loganiaceae	Pf W2 CR	0,3	18-hydroxyisosingucine	0,09	0,03	0,0001	0,2	23
<i>Tapirira guianensis</i> Aubl.	Anacardiaceae	Pf F32 CR	18	Cyclic alkyl polyol derivatives	4,7	2,7	0,49	18,9	24
<i>Tephrosia elata</i> Deflers	Fabaceae	Pf D6 CS	8,4	Elatadihydrochalcone	2,8	0,2	0,02	1,1	25
<i>Tephrosia elata</i> Deflers	Fabaceae	Pf D6 CS	8,4	Obovatin	4,9	0,05	0,004	0,2	25
<i>Tephrosia elata</i> Deflers	Fabaceae	Pf D6 CS	8,4	Obovatin methyl ether	3,8	0,01	0,001	0,03	25
<i>Tephrosia elata</i> Deflers	Fabaceae	Pf D6 CS	8,4	Deguelin	6,3	0,01	0,001	0,02	25
<i>Teucrium ramosissimum</i> Desfontaines	Lamiaceae	Pf FCB1	2,7	Homalomenol	1,2	0,04	0,001	0,2	26
<i>Tithonia diversifolia</i> (Hemsl.) A. Gray	Asteraceae	Pf FCA20 Ghana CS	0,75	Tagitinin (toxic)	0,33	2,7	0,02	11,6	27
<i>Toddalia asiatica</i> (L.) Lam.	Rutaceae	Pf K39 CS	22	Coumarin	16,2	2,0	0,44	5,3	28
<i>Vernonia brasiliiana</i> L.	Asteraceae	Pf BH2 CR	50	Lupeol	25	0,4	0,22	1,7	29
<i>Vernoniopsis caudata</i> (Drake) Humbert	Asteraceae	Pf FCB1 CR	1,6	Helenalin-[2-(1-hydroxyethyl)acrylate]	0,37	0,1	0,002	0,9	30
<i>Vernoniopsis caudata</i> (Drake) Humbert	Asteraceae	Pf FCB1 CR	1,6	Helenalin-[[2-(hydroxyethyl-3-methyl)acrylate]	0,07	0,01	0,0002	0,5	30
<i>Vernoniopsis caudata</i> (Drake) Humbert	Asteraceae	Pf FCB1 CR	1,6	11R,13-dihydrohelenalin-[2-(1-hydroxyethyl)acrylate]	0,15	0,02	0,0003	0,4	30
<i>Viola verecunda</i> A. Gray	Violaceae	Pf FCB1 CR	25	Epioleanolic acid	0,18	0,03	0,01	7	31
<i>Zanthoxylum rhoifolium</i> Lam.	Rutaceae	Pf FCB1 CR	10	Nitidine	1,8	6,00	0,6	50	32
<i>Zhumeria majdae</i> Rech.f. & Wendelbo	Lamiaceae	Pf W2 CR	7,5	12,16-dideoxy aegyptinone B	1,4	0,6	0,05	6,2	33

the compound at the  $IC_{50}$  of the extract. For the simplest case, the values are set such that  $\max=100$  and  $\max\text{-min}=100$  and  $\text{slope}=1$ . The calculated partial effect of various compounds appears in the column captioned “% of active compound at extract  $IC_{50}$ ”.

Taking for example the case of the crude extract of *Alstonia macrophylla* and one of the most active compounds, macrocarpamine: one obtains a yield for macrocarpamine of 0.95 % and it is straightforward to calculate that it is present in the extract at 0.054 mg at the  $IC_{50}$  of the extract. Using the above equation one gets  $f=16.7$ . Thus, the active compound contributes  $16.7/50$  of the overall effect or 33.4 %. Another active compound, villalstonine, contributes 31.1 % to the

activity of the crude extract. Given the fact that there are other active compounds in the extract, it is possible to suggest that the effects of macrocarpamine and villalstonine are not synergized in the crude extract and that their effects are additive. In such cases it can be concluded that very few active compounds account for the activity of the crude extract.

However, in the case of *Garcinia cowa* and 7-0-methylgarcinone, 0.0003 mg of the compound was present in the extract at the  $IC_{50}$  of the extract. The calculated  $f\sim 0$  and the compound contributes only 0.02 % to the anti-plasmodial activity of the crude extract. Since for all other purified compounds (cowanin, cowanol, cowaxanthone and b-mangostin) the contributions

are  $\leq 3.0\%$ , one is inclined to suggest that a strong synergism must occur between the components. Alternatively and quite unlikely, the extraction procedure destroys *all* the active compounds. In the extreme case of *Pothomorphe peltata* all the activity of the extract is accounted for by the activity of 4-nerolidylcatechol.

Inspection of the values that appear in the column captioned “% of active compound at extract IC<sub>50</sub>”, reveal that all cases can be subdivided in two groups. In one, the contribution of active compound to extract inhibition is  $\geq \sim 20\%$ , while in the second the values center around  $\sim 1\%$  or significantly lower. Thus, in the second group considerable synergism between active compounds must exist in order to account for the activity of the extract, or the extraction procedure (quite unlikely) destroys the activity of all compounds.

Among the hundreds of articles describing the antiplasmodial activity of plant extracts (1,031 articles were retrieved from PubMed for the last 10 years), only very few included the activity of the whole extract and of the pure compounds and their respective yields of extraction. Nevertheless it is striking that for 90% of the plants compiled in Additional file 1 the anti-malarial activity of the purified compounds cannot account quantitatively with that of the crude extract. If indeed this observation reflects the reality of anti-malarial properties of plant extracts, may be research should be focused on the “drug beside the drug”, looking for structures perhaps not exciting in the chemical point of view but that can revolutionize the treatment of malaria. Another natural consequence of this analysis is that evolution has provided not only bioactive metabolites that plants use to fight their foes, but has also mixed them in a very auspicious combination of compounds, which in some cases also work well in mammals. To achieve a similar combination even by systematic bioguided mixing is a very tedious, lengthy and expensive procedure. Why not learn from nature and optimize the use of plant extracts?

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#### Competing interests

The authors declare that they have no competing interests.

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