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 IC50 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 IC50 values and the yield of the purified compound are used. Data are shown in table 1.

The equation describing the relationship between concentration and IC50 is:

\[ f = \frac{\text{max} - (\text{max} - \text{min})}{(1 + x/EC_{50})^{\text{shape}}} \]

where “f” is the inhibitory effect. The EC50 is the IC50 of the isolated compound and “x” is the yield-dependent calculated concentration of...
| Plant Species                | Family     | Parasite strain | Extract IC₅₀ μg/ml | Most active compounds* | Compounds IC₅₀ μg/ml | Yield % | % of active comp of extract IC₅₀ | Contribution of active compound to extract inhib % | ref # |
|-----------------------------|------------|-----------------|-------------------|------------------------|----------------------|---------|---------------------------------|-----------------------------------------------|-------|
| Alstonia macrophylla Wall.  | Apocynaceae| Pf K1 CR        | 5.7               | Macrocarpamine          | 0.27                 | 0.95    | 0.05                           | 33.4                                         | 1     |
| Alstonia macrophylla Wall.  | Apocynaceae| Pf K1 CR        | 5.7               | Villalstonine           | 0.17                 | 0.6     | 0.03                           | 31.1                                         | 2     |
| Artemisia indica Wild       | Asteraceae | Pf K1 CR        | 6.6               | Exigua flavanones       | 4.6                  | 0.15    | 0.01                           | 0.4                                          | 3     |
| Bruea javanica L. (Merr.)   | Simaroubaceae| Pf K1 CR      | 0.5               | Brucein                 | 0.005                | 0.002   | 0.00001                        | 0.4                                          | 4     |
| Cryptolepis sanguinolenta (Lindl.) | Apocynaceae| Pf K1 CR      | 5.41              | Cryptolepine            | 0.054                | 0.04    | 0.002                          | 7.7                                          | 5     |
| Diospyros sanzaminika A. Chevalier | Ebenaceae | Pf K1 CR        | 0.8               | 4-O-(3′-methylgalloyl) norbergenin | 0.6                  | 1.2     | 0.01                           | 3.1                                          | 6     |
| Entyntina fusca Lour.       | Fabaceae   | Pf K1 CR        | 7.5               | Citiflavanone           | 5                    | 0.1     | 0.01                           | 0.4                                          | 7     |
| Entyntina fusca Lour.       | Fabaceae   | Pf K1 CR        | 7.5               | Lorchocarpol            | 1.6                  | 0.2     | 0.02                           | 1.9                                          | 7     |
| Entyntina fusca Lour.       | Fabaceae   | Pf K1 CR        | 7.5               | 8-Prenylaidzein         | 3.9                  | 0.0006  | 0.00005                        | 0.02                                         | 7     |
| Garcinia cowa L.            | Clusiaceae | Pf T9/94CS      | 5                  | 7-O-Methylgarcinone     | 2.5                  | 0.01    | 0.00030                        | 0.02                                         | 8     |
| Garcinia cowa L.            | Clusiaceae | Pf T9/94CS      | 5                  | Cowanin                 | 3                    | 0.2     | 0.01                           | 0.7                                          | 8     |
| Garcinia cowa L.            | Clusiaceae | Pf T9/94CS      | 5                  | Cowanol                 | 1.6                  | 0.5     | 0.03                           | 3.1                                          | 8     |
| Garcinia cowa L.            | Clusiaceae | Pf T9/94CS      | 5                  | Vowaxanthone            | 1.5                  | 0.4     | 0.02                           | 2.6                                          | 8     |
| Garcinia cowa L.            | Clusiaceae | Pf T9/94CS      | 5                  | b-Mangostin             | 3                    | 0.04    | 0.002                          | 0.1                                          | 8     |
| Geissospermum sencenium Miers | Rubiaceae  | Pf K1 CR        | 1.78              | Flavopereirine          | 2.84                 | 0.04    | 0.0008                         | 0.06                                         | 9     |
| Gomphostemma niveum Hook. f.| Lamiaceae  | Pf MR-CO2 CS    | 9.7               | Gomphostenin            | 38.2                 | 0.5     | 0.05                           | 0.3                                          | 10    |
| Gomphostemma niveum Hook. f.| Lamiaceae  | Pf MR-CO2 CS    | 3.4               | Gomphostenin-A          | 3.4                  | 0.24    | 0.83                           | 39                                           | 10    |
| Guiera senegalensis J. F. Gmel. | Combretaceae| Pf W2 CR      | 4.45              | Harman (b-carboline)    | 3.29                 | 0.1     | 0.00445                        | 0.3                                          | 11    |
| Holostylis reniformis Duch. | Rubiaceae  | Pf BHz/66/86 CR | 0.7               | Lignan                  | 0.12                 | 0.4     | 0.003                          | 4.6                                          | 12    |
| Holostylis reniformis Duch. | Rubiaceae  | Pf BHz CR       | 0.7               | Lignan                  | 0.12                 | 4.5     | 0.03                           | 42                                           | 12    |
| Nauclea orientalis L.       | Rubiaceae  | Pf D6 CS        | 3                  | Oleanolic acid          | 4.6                  | 0.07    | 0.002                          | 0.08                                         | 13    |
| Phyllanthus niruri L.       | Euphorbiaceae| Pf CS         | 1.3               | Terpenes                | 1.3                  | 0.1     | 0.002                          | 0.3                                          | 14    |
| Piptadenia pervillei Varke (Entada pervillei Varke (R.Vig.)) | Fabaceae | Pf MCF29        | 3.7               | Catechin derivatives    | 0.4                  | 0.03    | 0.001                          | 0.6                                          | 15    |
| Piptadenia pervillei Varke (Entada pervillei Varke (R.Vig.)) | Fabaceae | Pf FeM29 CR     | 3.7               | Catechin derivatives    | 0.3                  | 0.1     | 0.004                          | 2.4                                          | 15    |
| Pleiocarpa mutica Benth.    | Apocynaceae| Pf K1 CR        | 16.7              | Pleiomutinine           | 3.2                  | 0.05    | 0.008                          | 0.5                                          | 16    |
| Polyalthia debilis (Piere) Finet & ganep | Annonaceae| Pf K1 CR        | 1.35              | Bis-dehydroaporphine    | 4.1                  | 0.16    | 0.002                          | 0.1                                          | 17    |
| Pothomorphe peltata L.      | Piperaceae  | Pf K1 CR        | 3.7               | 4-Nerolidylcatechol     | 0.21                 | 5.7     | 0.21                           | 100                                          | 18    |
| Quissa amara L.             | Simaroubaceae| Pf W2 CR      | 8.9               | Simalikalactone D       | 0.005                | 0.001   | 0.0001                         | 3.5                                          | 19    |
the compound at the IC$_{50}$ of the extract. For the simplest case, the values are set such that max=100 and max-min=100 and 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 $f=0$ 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

| 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) |
| Rhaphidophora decursiva Schott | Araceae | Pf W2 CR | 6,8 | Polysyrphorin | 0,37 | 0,00004 | 0,000003 | 0,001 | 20 |
| Rourea minor (Gaertn.) Alston | Connaraceae | Pf W2 CR | 2 | Rourinoside (glycoside) | 1,2 | 4 | 0,08 | 12,5 | 21 |
| Stephania pieri Diels | Menispermaceae | Pf W2 CR | 3 | Asimilobine | 0,4 | 0,3 | 0,008 | 3,7 | 22 |
| Stychnos icaja Baillon | Loganiaceae | Pf W2 CR | 0,3 | 18-hydroxyisosangucine | 0,09 | 0,03 | 0,0001 | 0,2 | 23 |
| Tapirina guianensis Aubl. | Anacardiaceae | Pf F32 CR | 18 | Cyclic allyl poloyl derivatives | 4,7 | 2,7 | 0,49 | 18,9 | 24 |
| Tephrosia elata Deflers | Fabaceae | Pf D6 CS | 8,4 | Elatadihydrochalcone | 2,8 | 0,2 | 0,02 | 1,1 | 25 |
| Tephrosia elata Deflers | Fabaceae | Pf D6 CS | 8,4 | Obovatin | 4,9 | 0,05 | 0,004 | 0,2 | 25 |
| Tephrosia elata Deflers | Fabaceae | Pf D6 CS | 8,4 | Obovatin methyl ether | 3,8 | 0,01 | 0,001 | 0,03 | 25 |
| Tephrosia elata Deflers | Fabaceae | Pf D6 CS | 8,4 | Deguelin | 6,3 | 0,01 | 0,001 | 0,02 | 25 |
| Teucrium ramosissimum Desfontaines | Lamiaceae | Pf FCB1 | 2,7 | Homalomenol | 1,2 | 0,04 | 0,001 | 0,2 | 26 |
| Tithonia diversifolia (Hems!) A. Gray | Asteraceae | Pf FCA20 Ghana | 0,75 | Tagitin (toxic) | 0,33 | 2,7 | 0,02 | 11,6 | 27 |
| Toddalia asiatica (L.) Lam. | Rutaceae | Pf K39 | 22 | Coumarin | 16,2 | 2,0 | 0,44 | 5,3 | 28 |
| Vernonia brasiliana L. | Asteraceae | Pf BH2 CR | 50 | Lupeol | 25 | 0,4 | 0,22 | 1,7 | 29 |
| Vemoniosps caudata (Drake) Humbert | Asteraceae | Pf FCB1 CR | 1,6 | Helenalin-[2-(1-hydroxyethyl)acrylate] | 0,37 | 0,1 | 0,002 | 0,9 | 30 |
| Vemoniosps caudata (Drake) Humbert | Asteraceae | Pf FCB1 CR | 1,6 | Helenalin-[2-hydroxyethyl-3-methyl acrylate] | 0,07 | 0,01 | 0,0002 | 0,5 | 30 |
| Vemoniosps caudata (Drake) Humbert | Asteraceae | Pf FCB1 CR | 1,6 | 11R,13-dihydrohelenalin-[2-(1-hydroxyethyl)lactate] | 0,15 | 0,02 | 0,0003 | 0,4 | 30 |
| Viola verecunda A. Gray | Violaceae | Pf FCB1 CR | 25 | Epioleanolic acid | 0,18 | 0,03 | 0,01 | 7 | 31 |
| Zanthoxyllum rhoifolium Lam. | Rutaceae | Pf FCB1 CR | 10 | Nitidine | 1,8 | 6,0 | 0,6 | 50 | 32 |
| Zhumeria majdae Rech.f. & Wendelbo | Lamiaceae | Pf W2 CR | 7,5 | 12,16-dideoxy aegyptionine B | 1,4 | 0,6 | 0,05 | 6,2 | 33 |
are ≤ 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$_{50}$”, reveal that all cases can be subdivided in two groups. In one, the contribution of active compound to extract inhibition is ≥ ~20 %, while in the second the values center around ~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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**References**

1. Kasempredub N, Kirby GC, Steele JC, Houghton PJ: Antiplasmodial activity of extracts and alkaloids of three *Alstonia* species from Thailand. *Planta Med* 1999, 65:690-694.

2. Wright CW, Allen D, Phillipson JD, Kirby GC, Warhurst DC, Massiot G, Le Men-Olivier L: *Alstonia* species: are they effective in malaria treatment? *J Ethnopharmacol* 1993, 40:41-45.

3. Changpen R, Thebtaranonth Y, Wanapuphatthamkul S, Yuthavong Y: Antimalarial principles from *Artmesia indica*. *J Nat Prod* 1998, 61:1146-1147.

4. O’Neill MJ, Bray DR, Boardman P, Chan KL, Phillipson JD, Warhurst DC, Peters W: Plants as sources of antimalarial drugs, Part 4: Activity of *Bucea javanica* fruits against chloroquine-resistant *Plasmodium falciparum* in vitro and against *Plasmodium berghei* in vivo. *J Nat Prod* 1987, 50:41-48.

5. Paulo A, Gomes ET, Steele J, Warhurst DC, Houghton PJ: Antimalarial activity of *Cryptoplepis sanguinolenta* alkaloids from leaves and roots. *Planta Med* 2000, 66:30-34.

6. Tangmouo JG, Ho R, Matheussen A, Lannang AM, Kongouem J, Messi BB, Maes L, Hostettmann K: Antimalarial activity of extract and norbergenin derivatives from the stem bark of *Diospyros sanza-minika* A. Chevalier (Ebenaceae). *Phytother Res* 2010, 24:1676-1679.

7. Khoemek P, Ichino C, Ishiyama A, Sekiguchi H, Namatame M, Ruangrungsri N, Saffah E, Kiyohara H, Otoguro K, Oomura Y, Yamada H: In vitro antimalarial activity of prenylated flavonoids from *Erythrina fusca*. *J Nat Prod* 2008, 62:217-220.

8. Likhitwattayavud K, Phadungcharoen T, Kungrai J: Antimalarial xanthones from *Garcinia cowa*. *Planta Med* 1998, 64:70-72.

9. Steele JC, Veitch NC, Kite GC, Simmonds MS, Warhurst DC: Indole and beta-carbolone alkaloids from *Geissospermum sericeum*. *J Nat Prod* 2002, 65:65-88.

10. Sathe M, Kaushik MP: Gomphostemone: two new antimalarial compounds from the leaves of *Gomphostemma niveum*. *Boor Med Chem Lett* 2010, 20:1312-1314.

11. Fiot J, Sanon S, Azas N, Mahiou V, Janien O, Angenot L, Balansard G, Ollivier E: Phytochemical and pharmacological study of roots and leaves of *Guiera senegalensis* J.F. Gmel (Combretaceae). *J Ethnopharmacol* 2006, 106:173-178.

12. de Andrade-Neto VF, da Silva T, Lopes LM, do Rosário VE, de Pilla Varotti F, Krettli AU: Antiplasmodial activity of aryltetralone lignans from *Indole and beta-carbolone alkaloids from Geissospermum sericeum*. *J Nat Prod* 2002, 65:65-88.

13. de Andrade-Neto VF, Pohlit AM, Pinto AC, Silva EC, Nogueira KL, Melo MR, Henrique MC, Amorim RC, Silva LF, Costa MR, Nunomura RC, Nunnemura SM, Alecrim WD, Alecrim MG, Chaves FC, Vieira PP: In vitro inhibition of *Plasmodium falciparum* by substances isolated from Amazonian antimalarial plants. *Mem Inst Oswaldo Cruz* 2007, 102:359-365.
19. Houël E, Bertani S, Boudy G, Deharo E, Jullian V, Valentin A, Chevalley S, Stei D. Quassinoid constituents of *Quassia amara* L. leaf herbal tea. Impact on its antimalarial activity and cytotoxicity. *J Ethnopharmacol* 2009, 126:114-118.

20. Zhang HJ, Tamez PA, Vu DH, Ghee TT, Nguyen VH, Le TX, Le MH, Nguyen MC, Do TT, Soejarto PJ, Pezzuto JM. Antimalarial compounds from *Rhiphidophora decursiva*. *J Nat Prod* 2001, 64:772-777.

21. He ZD, Ma CY, Tan GT, Sydara K, Tamez P, Southavong B, Bouamanivong S, Soejarto PJ, Pezzuto JM, Fong HH, Zhang HJ. Rourinoside and rouremin, antimalarial constituents from *Rourea minor*. *Phytochemistry* 2006, 67:1378-1384.

22. Likhitwitayawuid K, Angerhofer CK, Chai H, Pezzuto JM, Cordell GA, Ruangrungsi N. Cytotoxic and antimalarial alkaloids from the tubers of *Stephania pireni*. *J Nat Prod* 1993, 56:1468-1478.

23. Frédérich M, De Pauw MC, Llabrès G, Tits M, Hayette MP, Brandt V, Penelle J, De Mol P, Angenot L. New antimalarial and cytotoxic sungucine derivatives from *Strychnos icaja* roots. *Planta Med* 2000, 66:262-269.

24. Roumy V, Fabre N, Portet B, Boudy G, Acebey L, Vigor C, Valentin A, Mouli C. Four anti-protozoal and anti-bacterial compounds from *Tapirira guianensis*. *Phytochemistry* 2009, 70:305-311.

25. Muva LM, Yenesew A, Derese S, Heydenreich M, Peter MG, Akala HM, Eyase F, Waters NC, Mutar C, Kenko JM, Walsh D. Antiplasmodial β-hydroxydihydrochalcone from seedpods of *Tephrosia elata*. *Phytochem Let* 2009, 2:99-102.

26. Henchiri H, Bodo B, Deville A, Dubost L, Zourgui L, Raies A, Grellier P, Mambu L. Sesquiterpenoids from *Teucrium ramosissimum*. *Phytochemistry* 2009, 70:1435-1441.

27. Goffin E, Ziemons E, De Mol P, de Madureira Mdo C, Martins AP, da Cunha AP, Philippe G, Tits M, Angenot L, Frédérich M. In vitro antimalarial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin C. *Planta Med* 2002, 68:543-545.

28. Oketch-Rabah HA, Mwangi JW, Lisgarten J, Mberu EK. A new antiplasmodial coumarin from *Toddalia asiatica* roots. *Fitoterapia* 2000, 71:636-640.

29. Alves TM, Nagem TJ, de Carvalho LH, Krettli AU, Zani CL. Antiplasmodial triterpene from *Vernonia brasiliana*. *Planta Med* 1997, 63:554-555.

30. Ramanandraibe V, Martin MT, Rakotondramana DL, Mambu L, Ramanirahambisoa D, Labaied M, Grellier P, Rasoanaivo P, Frappier F. Pseudoguanolide sesquiterpene lactones from *Vernoniopsis caudata* and their in vitro antimalarial activities. *J Nat Prod* 2005, 68:800-803.

31. Moon HI, Jung JC, Lee J. Antiplasmodial activity of triterpenoid isolated from whole plants of *Viola* genus from South Korea. *Parasitol Res* 2007, 100:641-644.

32. Jullian V, Boudy G, Georges S, Maurel S, Sauvain M. Validation of use of a traditional antimalarial remedy from French Guiana, *Zanthoxylum rhoifolium* Lam. *J Ethnopharmacol* 2006, 106:348-352.

33. Moerin MR, Pawar RS, Khan SJ, Tekwani BL, Khan IA. Antileishmanial, antimalarial and cytotoxic activities of 12,16-dideoxy aegyptione B from *Zumeria majdae* Rech.f. & Wendelbo. *Phytother Res* 2008, 22:283-285.

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