Antiplasmodial Phenolic Compounds from *Piptadenia pervillei*

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Key words
- *Piptadenia pervillei*
- Fabaceae
- antiplasmodial activity
- (+)-catechin gallate

Abstract

Piptadenia pervillei Vatke (Fabaceae) was selected from a screening programme devoted to the search of naturally-occuring antimalarial compounds from plants of Madagascar. Bioassay-guided fractionation of the ethyl acetate extract of the leaves led to the isolation of four phenolic compounds, (+)-catechin (**1**), (+)-catechin 5-gallate (**2**), (+)-catechin 3-gallate (**3**) and ethyl gallate (**4**). Structures were determined by NMR and mass spectroscopy. Compounds **2** and **3** displayed the highest *in vitro* activity against the chloroquine-resistant strain FcB1 of *Plasmodium falciparum* with IC_{50} values of 1.2 μM and 1.0 μM, respectively, and no significant cytotoxicity against the human embryonic lung cells MRC-5 was measured (IC_{50} values > 75 μM). Five analogues (**5–9**) of (+)-catechin 5-gallate (**2**) were synthesized and evaluated for their antiplasmodial activity.

Introduction

Drug-resistant *Plasmodium falciparum* malaria is a major killer and one of the most important obstacles to world health, especially in the developing countries of Africa. Parasites have developed resistance to all currently used antimalarial therapeutics, and this has been a driving force and challenge for much work devoted to the elucidation of the mechanisms of this resistance [1]. In the absence of effective vaccines, impregnated bednets and chemotherapy will remain the two most relevant tools for controlling malaria. Referring to chemotherapy, the development of a new class of drug is an urgent matter. To this end, screening of plant extracts for antiplasmodial activity is a useful way for discovering new leads, and the combination of structure-based design, mechanism-based design and combinatorial synthesis would be one appropriate strategy to bring lead candidates into drug development [1], [2], [3], [4].

As part of our search for novel antimalarial agents from plants, a screening programme was undertaken on plants of Madagascar on the basis of their traditional uses as antimalarials or their endemicity in ecologically different regions [5]. In our preliminary screen, *Piptadenia pervillei* Vatke, renamed *Entada pervillei* Vatke (R.Vig.) was selected. This plant is endemic to Madagascar [6] and no traditional uses were reported. The ethyl acetate extract of the leaves was found to exhibit a high antiplasmodial activity (IC_{50} = 0.5 μg/mL on the chloroquine-resistant strain FcM29 of *P. falciparum*) as well as a cytotoxic effect on murine P388 leukemia cells (IC_{50} = 8.5 μg/mL) [5]. The resulting selectivity index value, SI = 17, defined as the ratio of cytotoxicity over the antiplasmodial activity, prompted us to further investigate this species. A literature search revealed no prior phytochemical or pharmacological studies on *Piptadenia pervillei*. The EtOAc extract of this plant was submitted to a series of bioassay-directed fractionations and the most potent antiplasmodial fractions were rechromatographed leading to the isolation of four phenolic compounds **1–4** (**Fig. 1**). In this paper, we report the isolation and structure elucidation of these molecules as well as their *in vitro* antiplasmodial and cytotoxicity activities. To attempt an investigation of the structure activity-relationship, some analogues of compound **2** were synthesized and evaluated for their antiplasmodial and cytotoxicity activities.

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Bibliography
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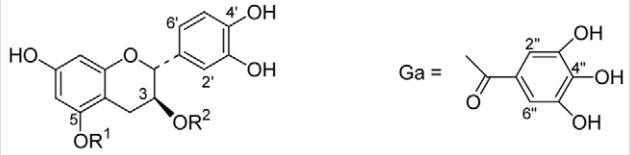

1 R^1 = H, R^2 = H
2 R^1 = Ga, R^2 = H
3 R^1 = H, R^2 = Ga

Fig. 1 Structures of compounds **1 – 3**.

Materials and Methods

General

The ^{1}H- and ^{13}C-NMR spectra were recorded at 599.915 and 150.93 MHz, respectively, on a Bruker AVANCE-600 spectrometer or at 400.13 and 100.61 MHz, respectively, on a Bruker AVANCE-400 spectrometer at 298 K, equipped with a ^{1}H-broadband reverse gradient probehead. Temperature was controlled by a Bruker BCU-05 refrigeration unit and a BVT 3000 control unit. Chemical shifts (δ_H) and (δ_C) are expressed in ppm relative to TMS with coupling constants (J) given in Hz. Mass spectra data were recorded with an electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in the positive and negative mode (QSTAR Pulsar I; Applied Biosystems). Optical rotations were conducted on a Perkin-Elmer 341 polarimeter at 20 °C. Analytical and preparative TLC were carried out on precoated Kieselgel 60 F_{254} plates (Merck). Spots were detected under UV (254 and 366 nm) before spraying with anisaldehyde-sulfuric acid solution in EtOH followed by heating the plate at 110 °C or with 2 % ethanolic ferric chloride reagent. Column chromatography was performed on 230 – 400 mesh silica gel 60 (Merck) and on Sephadex LH-20 (25 – 100 μm; Pharmacia Biotech Ltd). Preparative medium-pressure liquid chromatography (MPLC) was carried with a pump K-120 (Knauer) and Flasmart cartridges (silica gel 20 – 40 μm; AIT).

Plant material

Leaves of *Piptadenia pervillei* were collected in the eastern forest of Madagascar in August 2000. The plant material was identified by Armand Rakotozafy by comparison with an authentic specimen deposited at the Department of Botany of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. A voucher specimen has been kept at the Institut Malgache de Recherches Appliquées under the collection ID AR/MORA-014.

Extraction and bioassay-guided isolation

The air-dried powdered leaves of *P. pervillei* (200 g) were extracted three times with ethanol (EtOH) (500 mL, 3 × 24 hours) at room temperature, and the pooled ethanolic solutions were evaporated to dryness under reduced pressure to afford a crude extract. Partitioning of this extract between water and ethyl acetate (EtOAc) (3 × 500 mL) yielded a soluble organic extract, which displayed *in vitro* inhibitory activity against the chloroquine-resistant strain FcB1 of *P. falciparum* with an IC_{50} value of 3.7 μg/mL, whereas the water-soluble extract was devoid of antiplasmodial activity. Silica gel column chromatography of this extract (5 g) over 150 g (Ø column 40 mm, 25 mL fractions) with the solvent gradient CH_2Cl_2-MeOH gave 7 fractions. Three fractions, 3 (419 mg), 4 (850 mg) and 5 (1.83 g) showed significant antiplasmodial activities with IC_{50} values of 1.8 μg/mL, 1.4 μg/mL and 0.9 μg/mL, respectively. Purification of fraction 5 by column chromatography over silica gel (70 g) with CH_2Cl_2-MeOH-H_2O (80 : 20 : 5, 500 mL) yielded the active subfraction 53 with an IC_{50} value of 0.6 μg/mL (173 mg) which was subjected to column chromatography over Sephadex LH-20 eluted with MeOH-H_2O (70 : 30, 300 mL). Further separation of subfraction 532 (17 mg) on Sephadex LH-20 (MeOH-H_2O 85 : 15, 40 mL) furnished compounds **2** (5 mg) and **3** (1.5 mg) and on TLC, they gave both a strong blue dark spot with $FeCl_3$.

Successive treatment of fraction 4 on silica gel (34 g) with CH_2Cl_2-MeOH (93 : 7, 250 mL) and Sephadex LH-20 (MeOH-H_2O 70 : 30, 80 mL) afforded compound **1** (5 mg). Compound **4** (33 mg) was obtained from fraction 3 by column chromatography over silica gel (20 g) with cyclohexane-EtOAc (60 : 40, 210 mL).

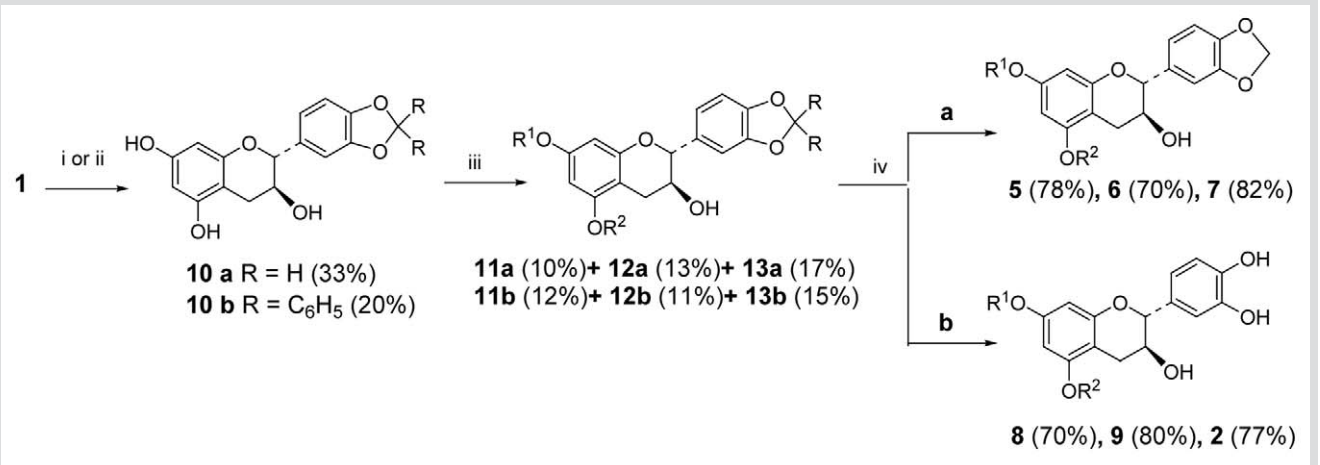

Fig. 2 Synthesis of the hemisynthetic derivatives. Reagents and conditions: (i) $BrCH_2Cl$, Cs_2CO_3, DMF, reflux, 33 %; (ii) $(C_6H_5)_2CCl_2$, Et_3N, CH_3CN, 0 °C to r.t., 20 h, 20 %; (iii) tri-*O*-benzylgalloyl chloride, DMAP, pyridine, r.t., 24 – 48 h, 10 – 17 %; (iv) H_2, 10 % Pd-C, MeOH or EtoAc, 12 – 24 h.

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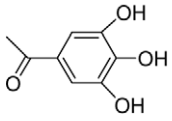
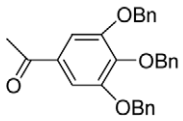
Table 1 Hemisynthetic derivatives[a]

Compounds	R	R^1	R^2
11a	H	H	Gp
12a	H	Gp	H
13a	H	Gp	Gp
11b	C_6H_5	H	Gp
12b	C_6H_5	Gp	H
13b	C_6H_5	Gp	Gp
5	–	H	Ga
6	–	Ga	H
7	–	Ga	Ga
8	–	Ga	H
9	–	Ga	Ga

[a] Ga =  Gp = 

Preparation of compounds 5–9

The analogues of compound **2** were synthesized from commercially available (+)-catechin (Sigma) as outlined in Fig. 2 (and Table 1). The catechol function was protected either as the methylene acetal with bromochloromethane (series a) and provided **10a** in 33% yield, or with the diphenylmethylene ketal group by treatment of **1** with dichlorodiphenylmethane (series b) affording **10b** in 20% yield [7], [8]. The reagent necessary for galloylation was obtained from gallic acid by benzylation with benzyl bromide followed by treatment with oxalyl chloride. Esterification of protected catechin (**10a**, **10b**) by tri-*O*-benzylgalloyl chloride in pyridine and in the presence of DMAP gave a mixture of three compounds for each series, **11**, **12**, **13**, in 10–17% yields [9]. The separation of these compounds was performed by flash chromatography on silica gel or by MPLC and the structure of each one was determined by 2 D NMR and MS analysis. Removal of the benzyl and diphenylmethylene groups was achieved by hydrogenolysis using 10% palladium on actived charcoal. Deprotection of **11b** gave a derivative of which the spectral data were identical to those of compound **2**. Compounds **5–7** were obtained without need of purification while compounds **8** and **9** had to be purified on Sephadex LH-20 to remove diphenylmethane generated during the reaction. All final compounds displayed spectral data (NMR, MS) which were consistent with the assigned structures.

In vitro antiplasmodial test

The *in vitro* antiplasmodial tests, based on the inhibition of [^{3}H]-hypoxanthine uptake by *Plasmodium falciparum* cultured in human blood, were performed as previously described [10]. Chloroquine diphosphate (Sigma Aldrich Chimie SARL) was used as positive control of the antiplasmodial activity.

In vitro cytotoxicity test on mammalian cells

Cytotoxicity was evaluated using human diploid embryonic lung cell line MRC-5. Cells were obtained from ATCC. They were maintained for 5 days in culture in the presence of drug and the cytotoxicity was determined using the colorimetric MTT assay according to the manufacturer's recommendations (cell proliferation kit I, Roche Applied Science). The concentration causing 50% of growth inhibition (IC_{50}) was obtained from the drug concentration-response curve, and the results were expressed as the mean from 3 independent experiments. Camptothecin (Sigma Aldrich Chimie) was used for comparison as standard drug of cytotoxic activity.

(+)-*Catechin 5-O-gallate* (**2**): ^{1}H-NMR (600 MHz, CD_3OD): δ = 7.16 (s, 2H, H2″, H6″), 6.81 (d, *J* = 1.6, H-6′), 6.73 (d, *J* = 8 Hz, 1H, H-5′), 6.70 (dd, *J* = 2, 8 Hz, H-2′), 6.23 (d, *J* = 2.2 Hz, 1H, H-8), 6.20 (d, *J* = 2.2 Hz, 1H, H-6), 4.61 (d, *J* = 7.4 Hz, 1H, H-2), 3.95 (m, 1H, H-3), 2.72 (dd, *J* = 8.2, 16 Hz, 1H, H-4b), 2.46 (dd, *J* = 5.2, 16 Hz, 1H, H-4a); ^{13}C NMR (150.75 MHz, CD_3OD): δ = 166.6 (CO), 158.3 (C-7), 157.2 (C-9), 151.9 (C-5), 146.9 (C-5″, C-3″), 146.5 (C-3′, C-4′), 140.1 (C-4″), 131.9 (C-1′), 120.4 (C-1″), 120.1 (C-6′), 116.2 (C-5′), 115.3 (C-2′), 110.7 (C-2″, C-6″), 106.6 (C-10), 103.6 (C-6), 101.7 (C-8), 83.2 (C-2), 68.3 (C-3), 28.8 (C-4).

Results and Discussion

By bioassay-guided fractionation, four phenolic compounds **1–4** were isolated. Compound **1** was identified as (+)-catechin by comparison of its spectral data with those published in the literature [11].

Compound **2** isolated as an amorphous powder exhibited a quasi-molecular ion $[M+H]^+$ at *m/z* = 443 in the ESI-TOF-MS spectrum in agreement with the molecular formula $C_{22}H_{18}O_{10}$. Interpretation of the COSY, HMQC and HMBC spectra demonstrated **2** to be a derivative of catechin **1**. The chemical shift pattern of 7 additional ^{13}C signals with reference to compound **1**, together with the presence of the singlet at δ = 7.16 accounting for two protons, indicated the presence of a galloyl unit in compound **2**. The upfield shift of the C-5 signal and the downfield shift of C-6, C-8 and C-10 signals with respect to compound **1** strongly suggested that the galloyl moiety was linked to C-5. Long-range correlations between H-6 and H-4 and C-5 were in agreement with our hypothesis. The typical downfield shifts due to esterification indicated the position of the carbon linked with the galloyl group. However, the NMR spectral data of compound **2** were also similar to those of (+)-catechin7-*O*-gallate isolated from *Sanguisorba officinalis* with inversion at C-5 and C-7 [12]. In this previous work, the structure was elucidated only by using 1 D NMR data and chemical modification. To definitively establish the position of the galloyl group, NMR spectra were performed in deuterated DMF at 230 K, which, unlike those performed in DMSO-*d6*, allowed us to distinguish a free hydroxy group (OH-3) at δ = 5.69 and three free phenolic hydroxy groups at δ = 9.70, 10.24 and 10.65, respectively. The observation of a correlation between the phenolic hydroxy group at δ = 10.65 and H-8 in the NOESY spectrum confirmed its presence in position 7, and subsequently allowed us to assign the galloyl group to position 5. On the basis of all the above evidence, compound **2** was determined to be (+)-catechin 5-*O*-gallate known as (+)-catechin 5-gallate [13].

Preliminary interpretation of the NMR data of compound **3** indicated that it was an isomer of compound **2** and was a flavonoid closely related to catechin **1**. Thus, when ^{1}H-NMR spectrum of compound **3** was compared to that of compound **1**, there was a downfield shilft of H-3 (+δ 1.39), along with the appearance of a singlet accounting for two protons at δ = 6.95. These preliminary observations suggested that compound **3** might be a catechin derivative esterified by gallic acid at C-3. The structure of compound **3** was definitively assigned as (+)-catechin 3-*O*-gallate known as (+)-catechin 3-gallate on the basis of comparison of its spectroscopic data with those given in literature [11].

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Table 2 *In vitro* antiplasmodial and cytotoxicity activities of compounds **1 – 9**

Compound	Antiplasmodial test, IC_{50} ± SD (μM)[a]	Cytotoxicity test, IC_{50} (μM)[b]	Selectivity Index (SI)
1	75.9 ± 4.6	> 75	> 1
2	1.2 ± 0.2	> 75	> 62
3	1.0 ± 0.2	> 75	> 75
4	8.1 ± 0.6	> 75	> 9
5	6.4 ± 1.1	ND	ND
6	18.7 ±2.2	ND	ND
7	6.6 ± 0.5	55.3 ± 3.6	8.4
8	16.3 ± 2.3	43.4 ± 7.9	2.7
9	10.2 ± 1.5	19.0 ± 3.9	1.9
Chloroquine	0.12 ± 0.04	ND	ND
Camptothecin	ND	0.0035 ± 0.0009	ND

[a] Chloroquine-resistant strain FcB1 was used for the antiplasmodial test.

[b] The cytotoxicity test was evaluated against MRC-5 cells.

ND: not determined. All experiments were realized in triplicate. Selectivity index is defined as the ratio of IC_{50} value determined on MRC-5 cells over the IC_{50} on *P. falciparum*.

Compound **4**, an amorphous powder, exhibited an $[M + H]^+$ ion peak at $m/z = 199$ compatible with the molecular formula $C_9H_{10}O_5$ as established by the positive ion TOF-MS. This compound was unambiguously assigned as ethyl gallate. It was probably an artifact produced by esterification of gallic acid, which occurs with alcoholic solvents during the extraction process [14].

The *in vitro* antiplasmodial activity of compounds **1 – 4** are shown in Table 2. (+)-Catechin 3-gallate (**3**) and (+)-catechin 5-gallate (**2**) were found to be the most active against the chloroquine-resistant strain FcB1 of *P. falciparum* with IC_{50} values of 1.2 μM and 1.0 μM, respectively. Ethyl gallate (**4**) was less active with an IC_{50} value of 8.1 μM. Catechin **1** showed no significant activity. The antiplasmodial activity of compounds **2**, **3** and **4** appears thus associated with the presence of a gallate ester which plays an important role in inhibition against *P. falciparum*. Compounds **2** and **3** exhibited comparable potency and the position of the galloyl group either at 3 or 5 does not seem to influence the activity. No significant cytotoxicity was measured on the human embryoic lung cells MRC-5 for these compounds (IC_{50} > 75 μM) and interesting selectivity towards *P. falciparum* was recorded (SI > 60). Work on phenolic compounds has shown that gallic acid esters and also catechin gallate esters had interesting anticancer properties, which seem to be caused by their antioxidant character [15], [16]. Some authors suggested that the formation of reactive oxygen species might be involved in the gallic acid induced apoptotic cell death but the mechanism of action remains uncertain [17]. Their ability to inhibit growth of other parasites was also tested and high activity was observed for ethyl gallate and catechin 3-gallate against *Trypanosoma cruzi* and for catechin 3-gallate against *Leishmania donovani* [18], [19].

A previous investigation on catechin **1** had shown that it was devoid of activity against *P. falciparum* and this was confirmed in this work [20]. Catechin and propyl gallate had only been reported to suppress the erythrocytic oxidative damage generated during infection by *Plasmodium berghei* on mice thanks to their antioxydant properties [21].

More recently, four catechins esterified with gallic acid at position C-3, namely (–)-epicatechin gallate, (–)-epigallocatechin gallate, (–)-catechin 3-gallate and (–)-gallocatechin gallate were reported to be potent inhibitors of β-ketoacyl-ACP-reductase (FabG), β-hydroxacyl-ACP-dehydratase (FabZ), and enoyl-ACP-reductase, enzymes involved in the type-II fatty acid biosynthesis (FAS-II) pathway of *P. falciparum,* with IC_{50} values on these enzymes in the range of 0.2 – 1.1 μM [22]. Among these catechin derivatives, (–)-catechin 3-gallate was the most active *in vitro* against *P. falciparum* with IC_{50} values of 0.4 and 3.2 μM, respectively, against the chloroquine-sensitive strain NF54 and the chloroquine-resistant strain K1. A similar range of inhibition was observed for this compound on the chloroquine-resistant strain FcB1 (IC_{50} value of 1 μM) in the present study. The other C-3 gallate ester derivatives showed moderate antiplasmodial activity (IC_{50}s between 6.2 and 40 μM). Finally, epigallocatechin gallate and epicatechin gallate have been found to potentiate the action of the antimalarial drug artemisinin [23].

To investigate the structure activity-relationship, hemisynthetic derivatives **5 – 9** (analogues of **2**) were prepared and their antiplasmodial activity evaluated *in vitro*. These compounds displayed moderate to weak activity against *P. falciparum* with IC_{50} values between 6.4 to 18.7 μM and weak to no significant activity against MRC-5 cells. Protection of 3′,4′-hydroxy groups (compound **5**) reduced the activity in comparison to compound **2**. Surprisingly, compounds **6**, **8** and **9** with the galloyl moiety in position 7 were less active than those with it in position 3 or 5 (compounds **2**, **3**, and **5**) suggesting probably that the proximity with a free hydroxyl or phenolic group may be favourable to the activity.

In summary, we have isolated and characterized four compounds including two catechin gallates (**2** and **3**), catechin (**1**) and ethyl gallate (**4)** from the leaves of *Piptadenia pervillei* by means of bioassay-guided fractionation. Access to further catechin gallate derivatives by synthesis showed that the best antiplasmodial activity was displayed when the catechol group is free and a galloyl moiety is present in the 3 or 5 positions.

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References

1 *Hyde JE.* Mechanisms of resistance of *Plasmodium falciparum* to antimalarial drugs. Microbes Infect 2002; 4: 165 – 74
2 *Brinner KM, Kim JM, Habashita H, Gluzman IY, Goldberg DE, Ellman JA.* Novel and potent antimalarial agents. Bioorg Med Chem 2001; 10: 3649 – 61
3 *Sullivan DJ.* Theories on malaria pigment formation and quinoline action. Int J Parasitol 2002; 32: 1645 – 53
4 *Fitch CD.* Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. Life Sci 2004; 74: 1957 – 72
5 *Rasoanaivo P, Ramanitrahasimbola D, Rafatro H, Rakotondramanana D, Robijaona B, Rakotozafy A et al.* Screening extracts of Madagascan plants in search of antiplasmodial compounds. Phytother Res 2004; 18: 742 – 7
6 *Villiers JF.* Subfamily mimosoideae. In: Du Puy DJ, editor. The Leguminosae of Madagascar. Kew: Royal Botanical Gardens; 2002: 154 – 293
7 *Zelle RE, McClellan WJ.* A simple, high-yielding method for the methylenation of catechols. Tetrahedron Lett 1991; 32: 2461 – 4
8 *Cren-Olivé C, Lebrun S, Rolando C.* An efficient synthesis of the four mono methylated isomers of (+)-catechin including the major metabolites and of some dimethylated and trimethylated analogues through selective protection of the catechol ring. J Chem Soc Perkin Trans I; 2002: 821 – 30
9 *Tückmantel W, Kozikowski AP, Romanczyk LJ.* Studies in polyphenol chemistry and bioactivity. 1. Preparation of building blocks from (+)-catechin. Procyanidin formation. Synthesis of the cancer cell growth inhibitor, 3-*O*-galloyl-(*2R,3R*)-epicatechin 4*β*,8-[3-*O*-galloyl-(*2R,3R*)-epicatechin]. J Am Chem Soc 1999; 121: 12 073 – 81
10 *Frappier F, Jossang A, Soudon J, Calvo F, Rasoanaivo P, Ratsimamanga-Urverg S et al.* Bisbenzylisoquinolines as modulators of chloroquine resistance in *Plasmodium falciparum* and multidrug resistance in tumor cells. Antimicrob Agents Chemother 1996; 40: 1476 – 81
11 *Davies AL, Cai Y, Davies AP, Lewis JR.* ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. Magn Reson Chem 1996; 34: 887 – 90
12 *Tanaka T, Nonaka GI, Nishioka I.* 7-*O*-Galloyl-(+)-catechin and 3-*O*-Galloylprocyanidin B-3 from *Sanguisorba officinalis.* Phytochemistry 1983; 22: 2575 – 8
13 *Malan E.* Derivatives of (+)-catechin-5-gallate from the bark of *Acacia nilotica*. Phytochemistry 1991; 30: 2737 – 9
14 *Calixto JB, Santos ARS, Cechinel V, Yunes RA.* A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med Res Rev 1998; 18: 225 – 58
15 *Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E.* Why drinking green tea could prevent cancer. Nature 1997; 387: 561
16 *Fraga CG, Martino VS, Ferraro GE, Coussio JD, Boveris A.* Flavonoids as antioxidants evaluated by *in vitro* and *in situ* liver chemiluminescence. Biochem Pharmacol 1987; 36: 717 – 20
17 *Kayser O, Kiderlin AF, Croft SL.* Natural products as potential antiparasitic drugs. In: Atta-ur-Rahman, editor. Studies in natural products chemistry. Bioactive natural products (Part G). Amsterdam: Elsevier; 2002: 26, 779 – 848
18 *Paveto C, Güida MC, Esteva MI, Martino V, Coussio J, Flawià MM et al.* Anti-*Trypanosoma cruzi* activity of Green tea (*Camellia sinensis*) catechins. Antimicrob Agents Chemother 2004; 48: 69 – 74
19 *Kolodziej H, Kayser O, Kiderlen AF, Ito H, Hatano T, Yoshida T et al.* Proanthocyanidins and related compounds: antileishmanial activity and modulatory effects on nitric oxid and tumor necrosis factor-α-release in the murine macrophage-like cell line RAW 264. Biol Pharm Bull 2001; 24: 1016 – 21
20 *Dickii J, Njifutie N, Foyere JA, Basco L, Ringwald P. In vitro* antimalarial activity of limonoids from *Khaya grandifolia* C.D.C. (Meliaceae). J Ethnopharmacol 2000; 60: 27 – 33
21 *Srivastava P, Chandra S, Arif AJ, Singh C, Pandey VC.* Metal chelators/antioxidants: approaches to protect erythrocytic oxidative stress injury during *Plasmodium berghei* infection in *Mastomys coucha.* Pharmacol Res 1999; 40: 239 – 41
22 *Tasdemir D, Lack G, Brun R, Rüedi P, Scapozza L, Perozzo R.* Inhibition of *Plasmodium falciparum* fatty acid biosynthesis: evaluation of FabG, FabZ, and FabI as drug targets for flavonoids. J Med Chem 2006; 49: 3345 – 53
23 *Sannella AR, Messori L, Casini A, Vincieri FF, Bilia AR, Majori G et al.* Antimalarial properties of green tea. Biochem Biophys Res Commun 2007; 353: 177 – 81

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