Anti-malarial Activity Of Phenolic Acids Is Structurally Related

Prince A Fordjour
University of Ghana Noguchi Memorial Institute for Medical Research
Jonathan P Adjimani
University of Ghana Department of Biochemistry Cell and Molecular Biology
Bright Asare
University of Ghana Noguchi Memorial Institute for Medical Research
Nancy O Duah-Quashie
University of Ghana Noguchi Memorial Institute for Medical Research
Neils B Quashie (nquashie@noguchi.ug.edu.gh)
University of Ghana

Research

Keywords: Phenolic, Antimalarial activity, Malaria, parasites, sensitivity, antioxidant

Posted Date: April 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-21702/v1

Abstract

Background In the absence of an effective vaccine against malaria, chemotherapy remains a major option in the control of the disease. Then, the recent report of the emergence and spread of clones of Plasmodium falciparum resistant to available antimalarial drugs should be of concern as it poses a threat to disease control. Compounds whose pharmacological properties have been determined and touted for other disease can be investigated for antimalarial activity. Phenolic acids (polyphenols) have been reported to exhibit antioxidant, anticancer, anti-inflammatory, antiviral and antibiotic effects. However, information on their antimalarial activity is scanty. Phenolic acids are present in a variety of plant-based foods: mostly high in the skins and seeds of fruits as well as the leaves of vegetables. Systematic assessment of these compounds for antimalarial activity is therefore needed. Method Using the classical in vitro drug test, the antimalarial activities of five hydroxycinnamic acids, (caffeic acid, rosmarinic acid, chlorogenic acid, o-Coumaric acid and ferulic acid) and two hydroxybenzoic acids (gallic acid and protocatechuic acid) against 3D7 clones of Plasmodium falciparum was determined. Results Among the phenolic acids tested, caffeic acid and gallic acid were found to be the most effective, with mean IC 50 value of 17.73µg/ml and 26.59µg/ml respectively for three independent determinations. Protocatechuic acid had an IC 50 value of 30.08 µg/ml. Rosmarinic acid and chlorogenic acid, showed moderate antimalarial activities with IC 50 values of 103.59µg/ml and 105µg/ml respectively. The IC 50 values determined for ferulic acid and o-Coumaric acid were 93.36µg/ml and 82.23µg/ml respectively. Conclusion The outcome of this study suggest that natural occurring phenolic compounds have appreciable level of antimalarial activity which can be exploited for use through combination of actions/efforts including structural manipulation to attain an increase in their antimalarial effect. Eating of natural food products rich in these compounds could provide antimalarial prophylactic effect.

Background

Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium*. In 2016, 216 million cases of malaria were recorded worldwide with 445,000 deaths (WHO Malaria Fact sheet, 2018). Among the species that infect humans, *P. falciparum* is the most virulent (Snow et al., 2005). In the absence of an effective malaria vaccine, the main control strategy against the disease is chemotherapy which has helped to reduce mortality and morbidity (Pradines, 2002 WHO Fact sheet 2016). However a major impediment to the global eradication of the disease is the emergence and spread of parasite resistant to most of the available antimalarial drugs. This has necessitated the search for novel drugs that can counter parasite resistance to the antimalarial drugs (Olliaro and Wirth, 1997).

Malaria parasites require iron for vital cell functions, such as the coordination of the cellular contents for growth and survival. The hosts generate iron withholding defense mechanisms to reduce infection (Gordeuk et al., 1994). Studies have shown that iron chelation therapy is a possible treatment for parasitic diseases including malaria (Pradines, 2002). This is because mature red blood cells (RBCs) do not have a way of taking up iron and therefore parasites in these cells tend to depend on the degradation of the RBCs hemoglobin for the supply of this nutrient, thereby making them perfect target for

chemotherapeutic attack by iron chelators (Cabantchik, 1995). Iron is needed by all living organisms for DNA synthesis, electron transport and in energy metabolism. Iron chelators show their action by chelating iron from various sources which include transferrin iron, intracellular iron and extracellular iron (Heppner et al., 1988). The antimalarial action of iron chelators is determined by three different factors namely; iron binding capacity of the chelator, chelator entry into and exit from the cell after treatment (Cabantchik, 1995). Some polyphenols have the potential to be used as chelators to regulate physiological reactions involving iron and other transitional metals in the parasite (Blache et al., 2002; Elhabiri et al., 2006; Haslam, 1996).

Polyphenols are cyclic derivatives of benzene with one or more hydroxyl groups attached to the aromatic ring (Fig. 1). The main classes of polyphenols are the phenolic acids, flavonoids, stilbenes and lignans. Studies have shown that polyphenols can act as reactive oxygen species scavengers and pro oxidant metals chelators (Blache et al., 2002; Haslam, 1996). They act as antioxidants by iron chelation, oxygen quenching and singlet formation (Brown et al., 1998).

Phenolic acids (hydroxycinnamic acids and hydroxybenzoic acids) are a sub class of polyphenol compounds consisting of a benzene ring to which either a propenoic group or a hydroxyl group, is attached. They are known to possess several pharmacological properties, including the prevention of the development of chronic diseases such as cancer and type-2 diabetes (Kim et al., 2012). There is a possibility that they have antimalarial activity due to their antioxidant activity and possible iron chelating ability. Their antioxidant activity may depend on certain structural factors which include; the position and number of hydroxyl groups attached to the benzene ring, the binding site and the type of substituents (Sroka & Cisowski, 2003).

The major aim of this study was, to determine the antimalarial activity of phenolic acids. *In vitro* susceptibility of *P. falciparum* to these compound was determined and the generated IC_{50} and antimalarial activities of the compounds with regard to the chemical structural differences discussed. We herein discuss the antimalarial activities of the compounds vice averse their potential as novel antimalarial candidates and propose mechanisms for their action. The structure of the phenolic acids used in this study is shown in Fig. 1.

Methods

Preparation of media and parasite culture

Incomplete RPMI 1640 culture media supplemented with hypoxanthine and glucose were prepared as previously described by Quashie and colleagues [Quashie et al., 2013]. Complete RPMI 1640 contains $NaHCO_3$ and Albumax (Invitrogen). Chloroquine-sensitive 3D7 clones of *P. falciparum* were used for all the *in vitro* drug tests carried out in this study. The parasites were originally kept in continuous cultured in RPMI 1640 at a hematocrit of 5% in a gas-mix containing 92.5% N_2, 5.5% CO_2, 2% O_2. The culture which was maintained at 37^0C was monitored and sub-cultured whenever parasitemia was between 3–5%.

Drug dilutions and preparation of test plates

All the phenolic acid compounds used in this study were obtained from Sigma Aldrich. Stock solutions at 1 mg/ml were initially prepared for each compound. Compounds were dissolved in 100% dimethyl sulphoxide (DMSO). Chloroquine was first dissolved in 1.5 ml deionized water after which the solution was made up to 5 ml with absolute ethanol. The drug solutions prepared were used immediately or stored at -80 °C and used within a month. Stock solutions were further diluted in complete RPMI 1640 to the desired starting concentrations after which a two-fold serial dilution was performed in 96-well tissue culture plate to give ten concentrations points for the *in vitro* drug test. The concentration range for the test compounds ranged from 0.1563-10 mM. Chloroquine was used as a positive control at concentrations ranging from 7.8-2,000 nM. Once pre-dosed with the test compounds or chloroquine, the plates were kept at 4 °C until ready for use. Test plates were used within three days after preparation.

In vitro test of susceptibility of Plasmodium falciparum to phenolic acid compounds

Ninety microliters of parasite culture was pipetted into 96 well plates containing 10 µl of each concentration of the test compound to generate a final parasitaemia and hematocrit concentrations of 0.5% and 2% respectively. Wells containing only DMSO were included as controls.. The plate was placed in a modular incubator chamber and gassed (gas contains 92.5% N_2, 5.5% CO_2, 2% O_2). The chamber was placed in an incubator set at 37ºC for 72 hours. The effect of the compounds on the parasites was assessed using the SYBR Green1 method previously described by Johnson and colleagues (Johnson et al., 2007). In brief, after 72 hours of incubation, the test plate was removed and 100 µl Malaria SYBR Green 1 fluorescent (MSF) lysis buffer containing SYBR Green was added to each well and mixed thoroughly by gently tapping on the plate. The plate was covered with aluminium foil and incubated at room temperature in the dark for at least two hours. Fluorescence was then read on the prototype micro titer plate reader (MTPR) (QIAGEN).

Data analysis

The concentration of anti-malarial drug inhibiting parasite growth by 50% (IC_{50}) for each compound was estimated from a dose response curve by non-linear regression analysis using GraphPAD Prism. The program generated IC_{50} estimates with associated 95% confidence intervals (CI). Estimated values with insufficient precision based on the CI were discarded. The experiment was performed on three separate occasions, and the mean IC_{50} ± S.E.M for each test drug was calculated.

Results

All but one of the compounds tested in this study showed significant activities against the malaria parasites. The results for the *in vitro* test of the hydroxybenoic acids against 3D7 clone of *P. falciparum*

are shown in Fig. 3. Gallic acid showed the highest activity with an IC_{50} value of 17.73 µg/ml and Protocatechuic had an IC50 value of 30.08 µg/ml. For the hydroxycinnamic acids, Caffeic acid had the highest activity followed by rosmarinic acid, chlorogenic, ferulic acid and o-coumaric with IC_{50} values of 26.59 µg/ml, 103.59 µg/ml, 105.76 µg/ml, 93.36 µg/ml and 82.23 µg/ml respectively (Fig. 3). Chloroquine which was used as internal control, inhibited the growth of the parasite with an IC_{50} value of 12.76 nM.

Table 1 Summary of IC_{50} of Phenolic acids

Phenolic Acid Compound	Mean IC50 value
Gallic acid	17.73
Rosmarinic	103.59
Caffeic acid	26.59
o-coumaric	82.23
ferulic acid	93.36
chlorogenic	105.76
Protocatechuic	30.08
Quinic	NA

Discussion

Reports of resistance of *P. falciparum* to most available antimalarial drugs has created an urgent need for novel drugs discovery. Compounds with completely different chemical properties and modes of action from existing ones have been strongly recommended as potential anti-parasitic agents for overcoming drug resistance (Olliaro and Wirth, 1997). This study looked at the potential of phenolic compounds as antimalarials..

Overall, it was observed that the phenolic acids tested showed significant but different antimalaria activities. This observation confirms the assertion by Andjelkovic and colleague that phenolic acids possess antimalarial property attributable to their ability to chelate iron (Andjelkovic *et al.*, 2005). Of the hydroxycinnamic tested, caffeic gave the highest inhibitory activity. The two adjacent hydroxyl groups on the benzene ring of the act as hydrogen donors (Rice-Evans et al., 1996) and make the compound an iron chelator. We speculate that the anti-plasmodium activity of caffeic acid may be due to the fact that, its metal chelating ability is enhanced by the nucleophilic nature of the aromatic ring and the electron donating effects of the COOH—CH = = CH— group on the ring which stabilizes the C = C double bond by resonance.

Rosmarinic acid and chlorogenic acid also showed some level of antiplasmodium activity. Chlorogenic acid is an ester of caffeic acid and quinic acid and its iron chelating activity is known to be dependent on the hydroxyl groups on the phenol moiety of caffeic acid (Rice-Evans et al., 1996). The hydroxyl groups attached to the cyclohexane ring in quinic acid are not known to chelate iron. Rosmarinic acid; an ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid has two (2) catechol groups that could be involved in the

chelation of iron and this could probably explains why it had higher antiplasmodial activity than chlorogenic acid.

Ferulic acid also showed *in vitro* anti-parasitic activity. This probably is in line with the suggestion that the antioxidant efficiency of monophenols can rise or increase to a higher extent by one or two methoxy substitutions at the *ortho* position relative to their hydroxyl group (Cuvelier *et al*., 1992). Ferulic acid may form complexes with iron only in the presence of a nucleophile or halogen which forms a bond with the methyl group by causing the O-CH_3 bond to dissociate. The displacement of methyl group places a partial negative charge on the oxygen, thus allowing it to bind easily to iron (Keppler *et al*., 2000). It is also possible that the presence of the methoxy group lowers the negative charge density of the chelating origin (Danilewicz *et al*., 2003). The inability of the methyl group to be displaced easily to facilitate iron chelation could explain the lower anti-plasmodium activity of ferulic compared to caffeic acid, rosmarinic acid and chlorogenic acid.

The parasites showed lower sensitivity to *p*-Coumaric and *o*-Coumaric acids. This must be due to the fact that mono-hydroxycinnamic acids have a relatively reduced iron chelating ability. Even though they both are mono-hydroxycinnamic acids, chelating of iron is strongly influenced by the presence of ortho hydroxyl position rather than the para hydroxyl position. This further explains why *o*-Coumaric acid had slightly higher activity than p-Coumaric acid. The two hydroxybenzoic acids namely gallic acid and protocatechuic acid used in this study showed high antiplasmodial activity *in vitro*. Gallic acid had a higher inhibitory effect on the parasites than protocatechuic acid and this could be due to the presence of the galloyl group in gallic acid compared to the catechol group of the protocatechuic acid. The number and position of the hydroxyl groups to the benzene ring could be another reason for the relatively higher antiplasmodial activity observed for gallic acid when compared with protocatechuic acid.

The metal chelating ability of polyphenols is associated with the presence of ortho*o*-dihydroxy polyphenols, that is, molecules bearing catechol or galloyl groups (Khokhar & Apenten, 2003; Moran et al., 1997). Despite the trihydroxy-benzoic or galloyl structure of gallic acid, it showed a lower activity than caffeic acid which has a catechol group. Suggesting that the galloyl group by itself might have lower iron chelating ability (Khokhar & Apenten, 2003). A plausible reason could be due to the electron donating effects of the COOH—CH = = CH— group on benzene ring of caffeic acid, the presence of which according to Cuvelier and colleagues might stabilize the C = C double bond in the ring by resonance and therefore increases its iron chelating ability (Cuvelier et al., 1991).

Plasmodium falciparum appears not to be susceptible to Quinic acid *in vitro*. This could be due to the absence of a phenolic OH group in the compound. As a result the hydrogen is not easily displaced for iron binding to occur. Another reason could be that the cyclohexane ring present has no delocalized electrons to facilitate the release of hydrogen for iron binding (Kono et al., 1998).

Conclusion

From observations and analysis made in this study it could be concluded that polyphenols have substantial antimalarial activity which is dependent on their structure. Caffeic and Gallic acids which are known to have the high iron chelating ability exhibited the highest antiplasmodial activities among the test compounds. It is important to consider these compounds when designing iron chelation antimalarial agents. Consumption of diets rich in these compound must be encourage among the populace in malaria endemic countries as part of the strategies in the fight against malaria.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

Funding

None

Authors' contributions

PAF, JPA, NDQ, and NBQ conceived and designed the study. PAF and BA carried out the experiments and led the drafting of the manuscript. JPA, NDQ, and NBQ reviewed the draft manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

The authors wish to acknowledge the contributions of Mr Felix Zoiku, Mr Prince Donkor, Mr Philip Agyeman and Mrs Sena Matrevi of Noguchi Memorial Institute for Medical Research

References

Andjelkovic M., Camp, J. V., Meulenaer, D. B., Depaemelaere, G., Socaciu, C., Verloo, M., and Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food chemistry*. 98, 23-31

Barros, J., Serrani-Yarce, J. C., Chen, F., Baxter, D., Venables, B. J. and Dixon, R. A. (2016) “Role of bifunctional ammonia-lyase in grass cell wall biosynthesis”. *Nature Plants*. 2: 16050.

Beard, J. L. (2001) “Iron biology in immune function, muscle metabolism and neuronal functioning”. *Journal of Nutrition.* 131(2): 5685-5805

Blache, D., Durand, P., Prost, M., and Loreau, N. (2002). (+)-Catechin inhibits platelet hyperactivity induced by an acute iron load *in vivo*. Free Radical Biology and Medicine, 33, 1670–1680

Brown, J. E., Khodr, H., Hider, R. C., and Rice-Evans, C. A. (1998). Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties. Biochemical Journal, 330, 1173–1178

Cabantchik, Z. I., (1995) Iron Chelators as antimalarials: the biochemical basis of selective cytotoxicity. *Parasitology Today* 11, 74-78

Cuvelier, M-E., Richard, H. and Berset, C. (1991) “Comparison of the antioxidative activity of some acid-phenols: structure-activity relationship”. *Bioscience, Biotechnology and Biochemistry.* 56(2): 324-325

Danilewicz, J. C. (2003). Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: central role of iron and copper. *American Journal of Enology and Viticulture*, 54, 73–85

Elhabiri, M., Carrer, C., Marmolle, F., and Traboulsi, H. (2006). Complexation of iron (III) by catecholate-type polyphenols. Inorganica Chimica Acta, 360, 353–359.

Golenser, J., Tsafack, A., Amichai, Y., Libman, J., Shanzer, A. and Cabantchik, Z. (1995). Antimalarial action of hydroxamate-based iron chelators and potentiation of desferrioxamine action by reversed siderophores. *Antimicrobial Agents and Chemotherapy*, 39(1), pp.61-65.

Gordeuk, V. R., Brittenham G. and Thuma P. E. (1994). Iron chelation as a chemotherapeutic strategy: In the treatment of malaria. Bergeron and G. M. Brittenham (ed.).The development of iron chelators for clinical use. CRC Press, Boca, Fla, p 11-130

Haslam, E. (1996). Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *Journal of Natural Products*, 59, 205–215

Heppner, D. G., Hallaway P. E., Kontoghiorghes G. J., and Eaton J. W. (1988). Antimalarial properties of orally active iron chelators. *Blood* 72: 357-361

Johnson J.D, Dennull R.A, Gerena L, Lopez-Sanchez M, Roncal N.E, Waters N.C.(2007) Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. *Antimicrob Agents Chemother*. 2007 Jun;51(6):1926-33. Epub 2007 Mar 19.

Khokhar, S., & Apenten, R. K. O. (2003). Iron binding characteristics of phenolic compounds: some tentative structure–activity relations. *Food Chemistry*, 81, 133–140.

Kim, E. O., Min, K. J., Kwon, T. K., Um, B. H., Moreau, R. A., Choi, S. W. (2012) Antiinflammatory activity of hydroxycinnamic acid derivatives isolated from corn bran in lipopolysaccharide-stimulated Raw 264.7 macrophages*. Food Chem Toxicol.50 (5):1309–16.*

Kono, Y., Kashine, S., Yoneyama, T., Sakamoto, Y., Matsui, Y., Shibata, H. (1998) Iron Chelation by Chlorogenic Acid as a Natural Antioxidant. *Bioscience, Biotechnology, and Biochemistry, 62:1, 22-2*

Moran, J. F., Klucas, R. V., Grayer, R. J., Abian, J., & Becana, M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radical Biology and Medicine*, 22, 861–870.

Olliaro, P. and Wirth, D. (1997). New Targets for Antimalarial Drug Discovery. *Journal of Pharmacy and Pharmacology*, 49(S2), pp.29-33

Pradines, B. (2002). Iron chelators as antimalarial agents: in vitro activity of dicatecholate against *Plasmodium falciparum*. *Journal of Antimicrobial Chemotherapy*, 50(2), pp.177-187.

Quashie, N.B., Duah, N.O., Abuaku, B, Quaye L., Ayanful-Torgby, R., Akwoviah, G.A., Kweku, M., Johnson, J.D., Lucchi, N.W., Udhayakumar, V., Duplessis, C., Kronmann, K.C., Koram, K.A., (2013). A SYBR Green 1-based *in vitro* test of susceptibility of Ghanaian *Plasmodium falciparum* clinical isolates to a panel of anti-malarial drugs. Malar J. 12:450.

Reinke, L. A., Moore, D. R., Rau, J. M., & Mccay, P. B. (1995). Inorganic-phosphate promotes redox cycling of iron in livermicrosomes – effects on free-radical reactions. *Archives of Biochemistry and Biophysics*, 316, 758–764.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structureantioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, 933–956.

Snow, R.W., C.A. Guerra, A.M. Noor, H.Y. Myint, and S.I. Hay. 2005. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 434:214–217

Sroka, Z., & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology, 41, 753–758

Welch, K. D., Davis, T. Z., and Aust, S. D. (2002). Iron autoxidation and free radical generation: effects of buffers, ligands, and chelators. *Archives of Biochemistry and Biophysics*, 397, 360– 369.

WHO (2016) World Malaria Report 2016. Geneva, Switzerland: World Health Organization Wijesundera Mde S (1988) Malaria outbreaks in new foci in Sri Lanka*. Parasitol Today* 4: 147– 150

Figures

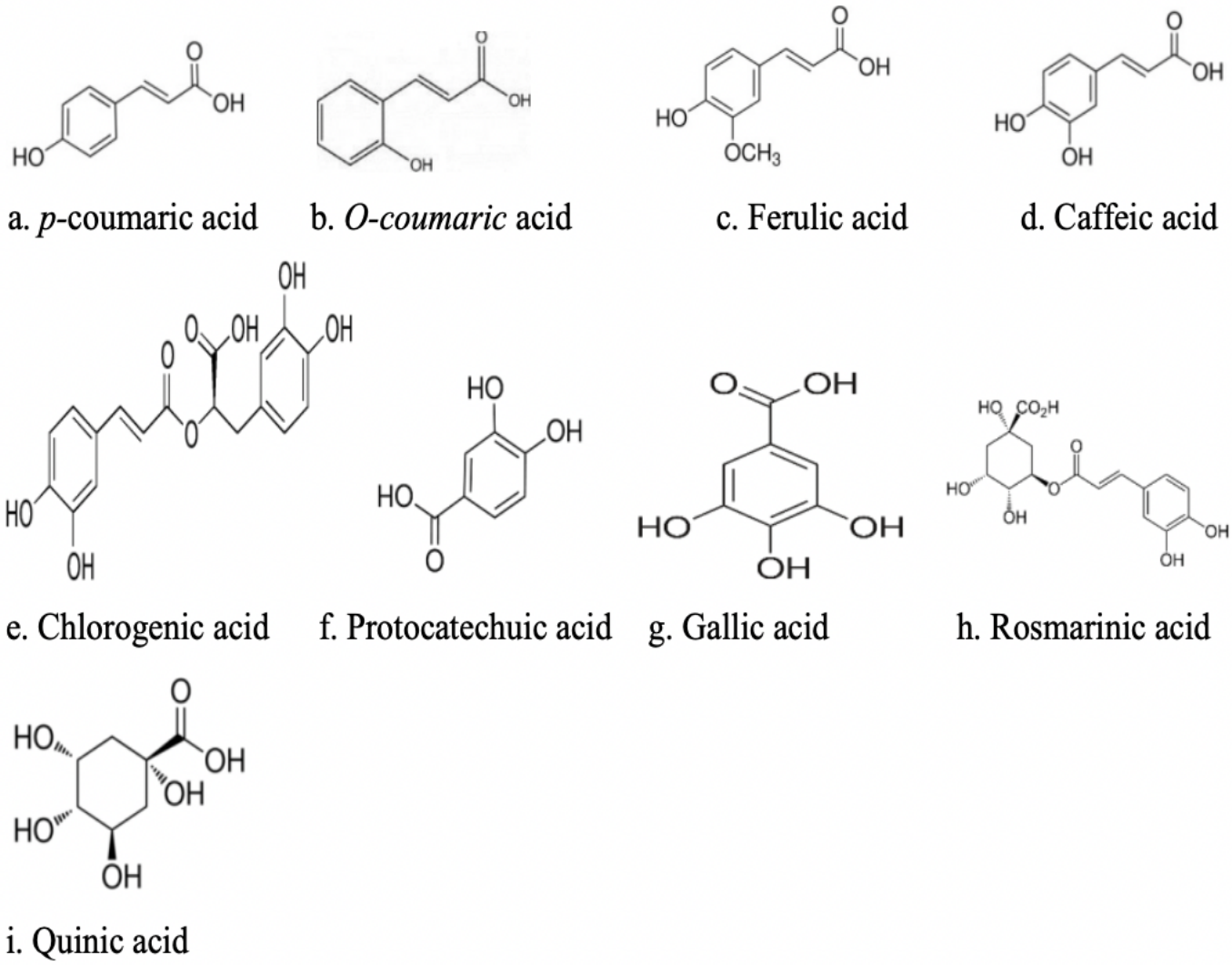

Figure 1

Structures of phenolic acids used in this study

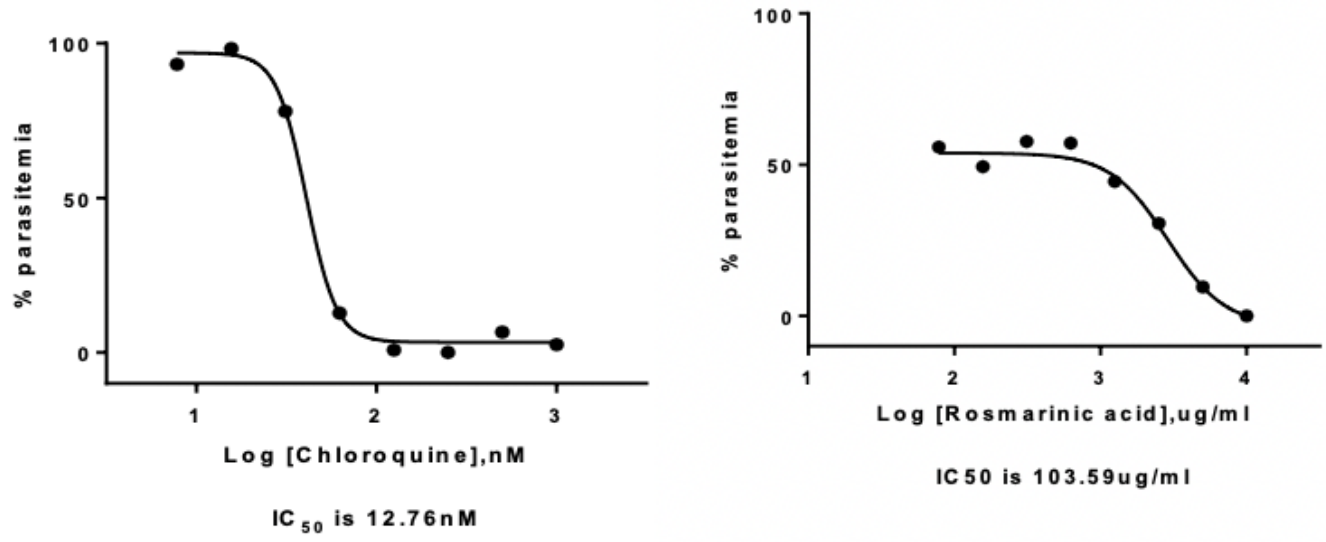

Figure 2

A representative dose-response curve, for chloroquine and rosmarinic acid

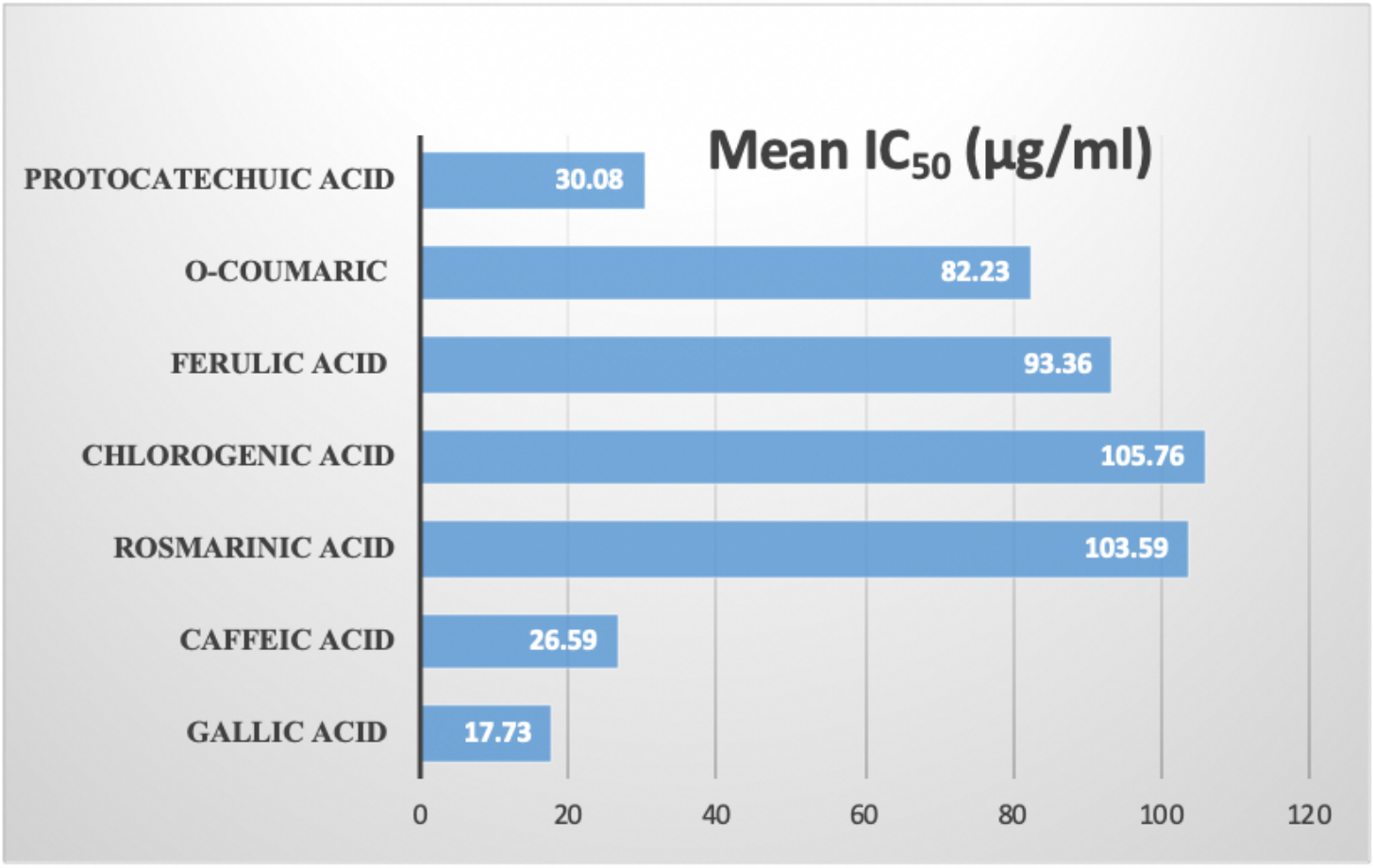

Figure 3

Susceptibility of P. falciparum to phenolic acid compounds in vitro.