MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

A REVIEW OF THE ANTIPARASITIC EFFECTS OF ELLAGIC ACID AND OTHER PHENOLIC COMPOUNDS ISOLATED FROM MEDICINAL PLANTS

Anjana Chamilka Thuduhenage Dona[1], Nurul Hammizah Hamidon[1,2], and Nurhidanatasha Abu Bakar[1*]

[1]*School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*
[2]*SIRIM Berhad, Persiaran Dato' Menteri, P.O. Box 7035, Section 2, 40700 Shah Alam, Selangor, Malaysia*

*Corresponding Author: natashaa@usm.my

REVIEW ARTICLE

History
Received: 19th March 2023
Accepted: 8th July 2023

Keywords:

Ellagic acid; Phenolic compounds; Plasmodium; Trypanosoma; Leishmania; Medicinal plants

Abstract

The pursuit of alternative sources for antiparasitic drugs is crucial due to the frequent inadequacy of current treatments and the potential development of resistance among parasites towards synthetic therapies. Conversely, medicinal plants have garnered prominent attention as it produces various natural compounds with intriguing biological properties that can be beneficial to treat parasitic infections. This article presents a comprehensive overview of past literature regarding the topic. It specifically focuses on the antiparasitic effects of ellagic acid and other phenolic compounds against parasites, such as *Plasmodium, Trypanosoma, Leishmania, and Helminths*. The findings hope to contribute further understanding on the utilisation of ellagic acid and other phenolic compounds as innovative antiparasitic drugs for effectively managing parasitic infections. It also highlights potential limitations and set the direction for future research.

INTRODUCTION

The global population has witnessed a widespread of parasitic diseases with approximately 1 to 2 billion infection cases and several million deaths being reported annually [1]. It is commonly caused by parasitic infections with notable examples include malaria, trypanosomiasis, leishmaniasis, and helminthiases [2]. Many antiparasitic drugs have been introduced as a countermeasure to such issue [3]; however, certain parasite strains have developed resistance to these drugs over time. This leads to the lack of effective drugs, subsequently posing a challenge for the prevention of parasite infections [2].

Medicinal plants have been traditionally used to treat parasitic infections. Past literature suggests that efforts to initiate the search for antiparasitic natural compounds should begin in Asia, Africa, and Latin America [2] as these nations have a greater number of publications on traditional medicine and possess a larger variety of medicinal plants compared to other nations [4]. It is feasible to discover novel bioactive components as medicinal plants abound in natural compounds with a wide range of structural diversity [5]. Ellagic acid and other phenolic compounds derived from plants have undergone frequent laboratory examinations [6, 7, 8] and are believed to hold the potential for antiparasitic actions [9, 10, 11].

Crude extracts from various parts of medicinal plants, such as *Punica granatum* roots and *Carica papaya* roots, leaves, and seeds, are believed to possess efficacy against various helminths [12]. Compounds like benzyl thiocyanate isolated from *C. papaya* seeds and diospyrol from *Diospyros mollis* fruits have demonstrated effectiveness against *Ascaris lumbricoides* and *Necator americanus,* respectively [12]. Extracts of *Lippia multiflora*, *Aframomum sceptrum*, and *Uvaria afzelii* have exhibited antileishmanial activity (IC_{50} = <25 µg/mL) [13]. The reference drug, miltefosine, demonstrated antimalarial activity with an IC_{50} of 8.219 ± 0.6337 µg/mL [14]. In Ivory Coast, *Lawsonia inermis* is specifically prescribed for the treatment of African trypanosomiasis [8]; however, no efficacy has been

demonstrated against other parasites. Extracts from *Terminalia glaucescens* and *Anogeissus leiocarpus* have also exhibited antimalarial activity (IC_{50} = <5 µg/mL) and are recommended by traditional healers for the prevention of malaria infections [13].

PHENOLIC COMPOUNDS

The primary products of plant secondary metabolism are phenolic compounds. Apart from protecting plants against parasites and insects, phenolic compounds are responsible for pigmentation, astringency, and UV light protection [15]. These substances are present in a wide variety of matrices, including fruits (blueberries and apples) and vegetables (potatoes and red cabbage) [16, 17]. In terms of molecular characteristics, phenolic compounds are characterised by one or more aromatic rings bonded to one or more hydroxyl substituents, resulting in diverse chemical structures [18]. These compounds are categorised into five groups: flavonoids, tannins, lignans, stilbenes, and phenolic acids.

Flavonoids

As shown in Figure 1A, flavonoids can be categorised into numerous subgroups based on their unsaturation and oxidation status (the carbon of the C ring is bonded to the B ring) [19]. In the case of isoflavones, the B ring is attached to position 3 of the C ring, while for neoflavonoids, the B ring is linked to position 4 of the C ring (Figure 1B-C). A variety of flavonoid subgroups have their B rings attached at position 2 of the C rings, including flavones, flavonols, flavanones, flavanonols, flavanols or catechins (flavan-3-ols), anthocyanins, and chalcones (Figure 1D-J) [19].

Several flavonoids have been extensively investigated and have demonstrated activity within the concentration range of 5-32 µM against *Cryptosporidium parvum* and *Entamoeba intestinalis* [20]. Specific flavonoid compounds, such as apigenin and genistein, are known as protein tyrosine kinase inhibitors and have exhibited inhibitory effects against both *C. parvum* and *Toxoplasma gondii* [21, 22]. Earlier studies reported that genistein, a protein tyrosine kinase inhibitor, impedes calcium ionophores from inducing the release of *Toxoplasma gondii* tachyzoites from host cells, including neutrophils or kidney cells [23]. Apigenin exerts its inhibitory action against *C. parvum* through the induction of host cell apoptosis [20]. Phenylpropanoid and Chromone flavonoids have demonstrated effectiveness against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* in vitro [24]. Additionally, flavonoids such as casticin and chrysosplentin are believed to hinder the production of fatty acids by parasites [25, 26, 27]. Notably, flavonoids have been identified as potent active agents in an in vitro assay targeting *Leishmania amazonensis* amastigotes. Among these, the flavonoid compound kaempferol 3,7-di-O-methyl ether exhibited significant efficacy against amastigotes, yielding the IC_{50} value of 10.5 ± 2.5 µM. Comparatively, previous research indicates that the commercial drug miltefosine holds the IC_{50} value of 18.933 ± 0.737 µM for the *Leishmania amazonensis* CU1 form, with effective IC_{50} values ranging from 3.88 to 28.7 (>306.6) µM [28, 29, 30]. Moreover, a distinct study revealed that quercetin, gallic acid, and rutin compounds also exerted effectiveness against *L. donovani*, yielding the IC_{50} values of 84.65 µg/mL, 86 µg/mL, and 98 µg/mL, respectively [31]. These compounds induced alterations in the ultrastructure of treated cells, ultimately leading to parasite demise. Further effects included the enhancement of nitric oxide generation, initiation of apoptosis, and DNA damage. Additionally, these compounds suppressed critical enzymes trypanothione reductase and trypanothione synthetase that are essential for leishmanial survival [31]. In a previous investigation, phenolic and flavonoid compounds within the plant extracts of *P. retrofractum* (IC_{50} = 87.63 ± 3.55 µg/mL), *A. gangetica* (IC_{50} = 39.06 ± 1.98 µg/mL), and *A. scholaris* (IC_{50} = 48.92 ± 1.52 µg/mL) demonstrated pronounced inhibitory activity against the proteolytic subunit of the caseinolytic protease system of *P. knowlesi* (Pk-ClpP) [32].

Tannins

Tannins refer to flavonoid polymers that can be classified into two subclasses: condensed tannins and hydrolysable tannins. Mixtures of simple phenols can give rise to hydrolysable tannins (Figure 2) [33]. Under the influence of acidic or basic conditions, these compounds can undergo hydrolysis, leading to the formation of phenolic acids and carbohydrate molecules. For example, gallic acid can serve as a precursor for hydrolysable tannins (Figure 2A). Condensed tannins are assembled through the linkage of two or more monomers of flavan-3-ol units, primarily connected via bonds between their A rings and the pyran rings of other flavanols (Figure 2B). Due to their intricate structural nature, tannins have not been extensively explored in terms of their bioactive potential [18]. However, proanthocyanidins have recently garnered attention due to their potential health benefits, including anti-inflammatory and antimicrobial activities [18, 34].

Distinct variations in activity have also been observed when goats were administered with condensed tannins before being exposed to third-stage larvae, resulting in reductions of worm populations by 33%, 70%, and 66% for *H. contortus, T. circumcincta, and T. colubriformis*, respectively [35]. Condensed tannins exhibit chelation properties for iron and zinc that are crucial for the growth and reproduction of *P. falciparum*. While the role of iron is better understood, the function of zinc is less recognised. The parasite-driven influx of poorly bound zinc into infected erythrocytes contributes to the parasite's pathogenicity. Prior to host cell rupture, the *P. falciparum* parasite must acquire 30 million zinc atoms, corresponding to a 400% increase in overall zinc concentration. Notably, highly zinc-specific

chelators have demonstrated the capability to arrest the parasite's development at the trophozoite stage while concurrently inhibiting parasite proliferation. The presence of sufficient chelator concentration renders the parasite nonviable [36]. Furthermore, the tannin-treated larvae of *A. suum* displayed notable effects with all larvae succumbing to treatment at a concentration of 1 mg/mL. Even at a concentration of 111 mg/mL, motility was greatly diminished. These findings strongly suggest that condensed tannins exert potent anthelmintic effects on both the L4 and L3 stages of *A. suum* [37].

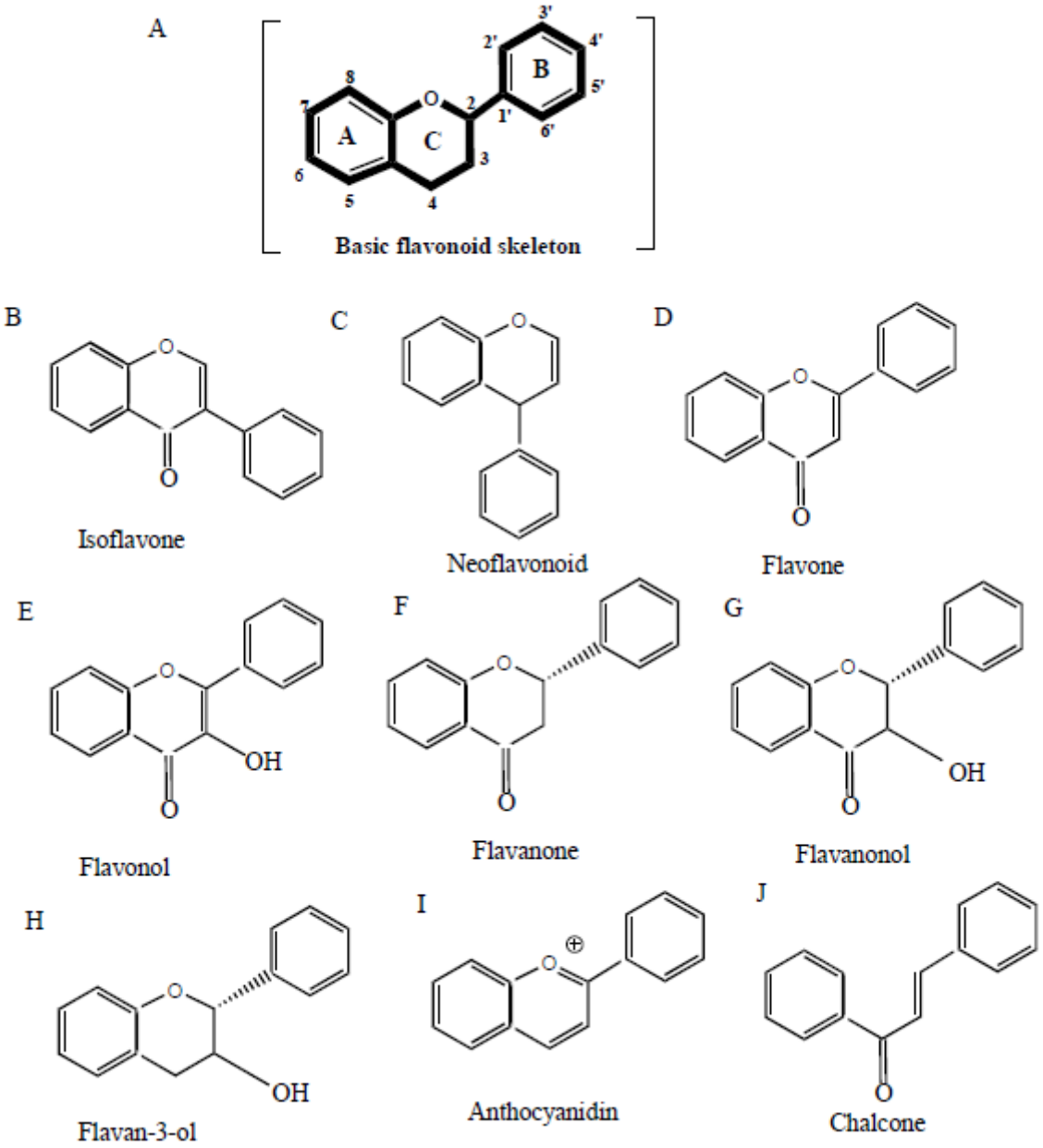


Figure 1. The basic chemical structure of flavonoids and their classes.

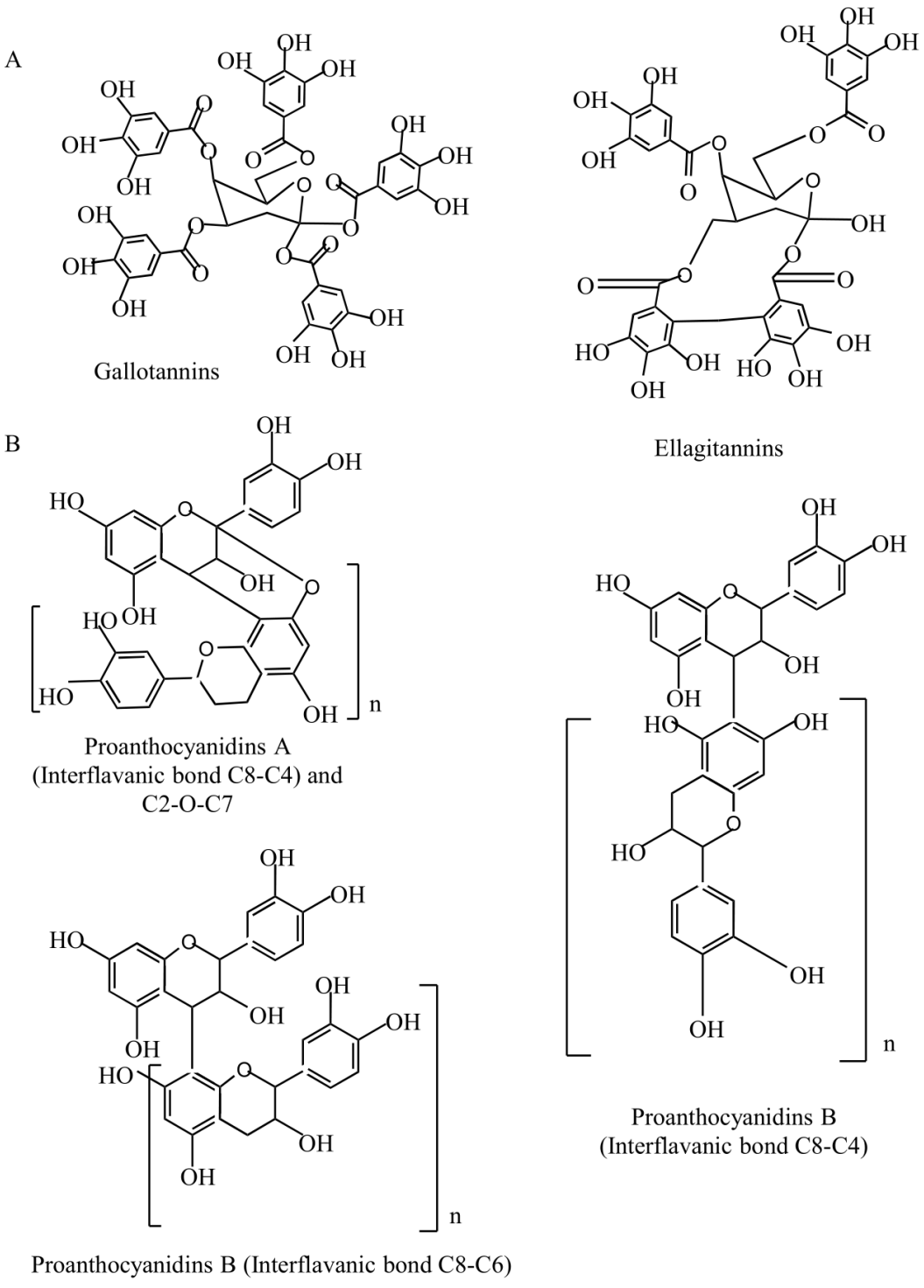


Figure 2. The chemical structure of tannins. (A) Hydrolysable tannins, which are allotannins and ellagitannins. (B) Condensed tannins, which are proanthocyanidins A, proanthocyanidins B and proanthocyanidins C.

Lignans

The fundamental structure of lignans consists of an amalgamation of phenylpropanoid dimers (C6-C3) that are interconnected through the central carbons of their side chains, as shown in Figure 3 [38]. Lignans represent secondary plant metabolites exhibiting diverse chemical configurations that are often found in specific seeds. It also exhibits both estrogenic and antiestrogenic properties than various other bioactivities [38]. Among the isolated tetrahydrofuran lignans, calopeptin demonstrated activity against *P. falciparum* D6 and W2 strains, yielding the IC_{50} values of 3800 ng/mL and 3900 ng/mL, respectively [39]. Notably, lignans machilin-G and veraguensin exhibited promising antileishmanial efficacy against *L. donovani*, yielding the IC_{50} values of 18 µg/mL and 36 µg/mL, respectively [39]. In terms of potency against *T. cruzi* trypomastigotes, both copalic acid and kusunokinin emerged as the most effective lignans when compared to fargesin, epieudesmin, and sesamin lignans. The reference drugs employed in the study included pentamidine, glucantim, and benznidazole [40]. Copalic acid displayed selectivity against parasitized erythrocytes and minimal toxicity towards other mammalian cells. Notably, kusunokinin showed substantial efficacy against trypomastigotes (IC_{50} = 51 µM) without inducing hemolytic activity [40].

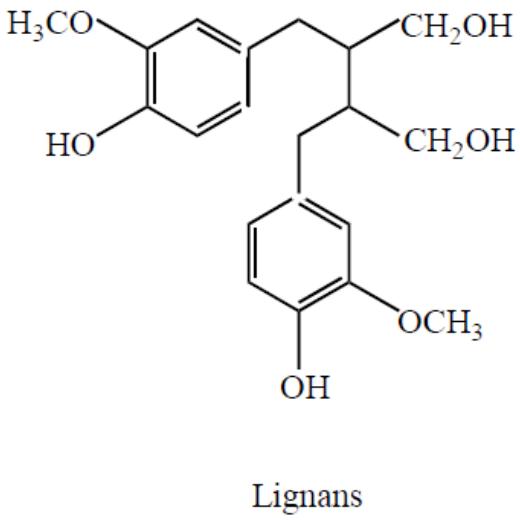


Figure 3. The chemical structure of lignans.

Stilbenes

The chemical structure of stilbenes, characterised by the presence of a double bond linking two benzene rings, is represented by the formula C6-C2-C6 (Figure 4 A) [18]. Stilbenes can be categorised into Z and E isomers based on the location of their central double bond. While over 400 stilbenes have been identified, only plant families possessing the pivotal enzyme necessary for stilbene biosynthesis can produce these compounds. Among the prominent stilbenes, resveratrol stands out (Figure 4 B) and has been extensively researched due to its notable bioactivities [18]. Stilbene-based compounds have demonstrated antiplasmodial action, inducing stage-specific apoptotic death in young Plasmodium parasites [41]. The occurrence of DNA fragmentation and loss of mitochondrial membrane potential serves as evidence of the parasite-specific programmed cell death initiated by stilbene-based compounds. Furthermore, stilbene ST18 has been reported to become active against *T. cruzi* amastigotes and exhibits lower cytotoxicity when evaluated in vitro on normal healthy cells [42]. It is theorised that stilbene ST18 activates the conserved enzyme caspase-1 in infected macrophages, which is an essential regulator of parasitemia, host survival, and the onset of the adaptive immune response in Trypanosoma infection.

Phenolic Acids

Plant phenolics exhibit numerous similarities with aliphatic alcohols [43]. Phenolics function as weak acids rather than strong alcohols due to their possession of an aromatic ring and a hydrogen atom within the hydroxyl group. These compounds are crucial for performing various bioactivities, including supporting plant development, growth, and defense, while also exerting positive health impacts on humans [44]. Phenolics are recognised natural antioxidants that play a significant role in various biological and pharmacological activities, such as anti-inflammatory,

anticancer, antimicrobial, antiallergenic, antiviral, antithrombotic, hepatoprotective, and antiparasitic [45, 46, 47, 48]. Two hydroxybenzoic acids (Figure 5) and five hydroxycinnamic acids (Figure 6) have been evaluated for their inhibitory potential against the growth of *P. falciparum* (3D7 clone) [49]. Both protocatechuic acid and gallic acid displayed the IC_{50} values of 30.08 µg/mL and 17.73 µg/mL, respectively. Additionally, caffeic acid, o-coumaric acid, chlorogenic acid, ferulic acid, and rosmarinic acid (Figure 6A-E) yielded the IC_{50} values of 26.59 µg/mL, 82.23 µg/mL, 105.76 µg/mL, 93.36 µg/mL, and 103.59 µg/mL, respectively [49].

Rosmarinic acid is proven to be more effective in dismantling the intracellular promastigote and amastigote forms of *L. donovani* [50]. The mechanism of action involves iron chelation, resulting in cell cycle arrest and various morphological alterations [50]. Past research investigated gallic acid in an anthelmintic bioassay to determine the time of paralysis and death in the earthworm *Pheretima posthuma*. It was found that the worm exposed to gallic acid exhibited dose-dependent paralysis and eventual demise [51]. Furthermore, gallic acid was tested for its antitrypanosomal activity against *T. brucei brucei* in vitro (IC_{50} = 14.2 ± 1.5 µM). This compound influenced both mitochondrial membrane integrity and cell viability in a dose-dependent manner. Moreover, gallic acid-induced kinetoplast loss in trypanosomes [52].

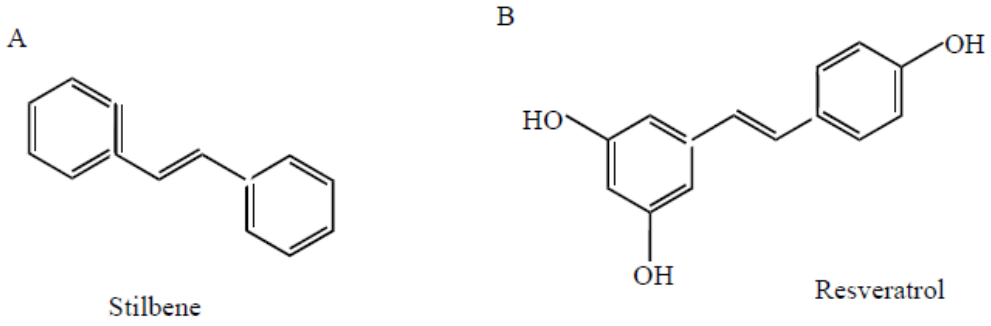


Figure 4. The chemical structure of (A) stilbene and (B) resveratrol.

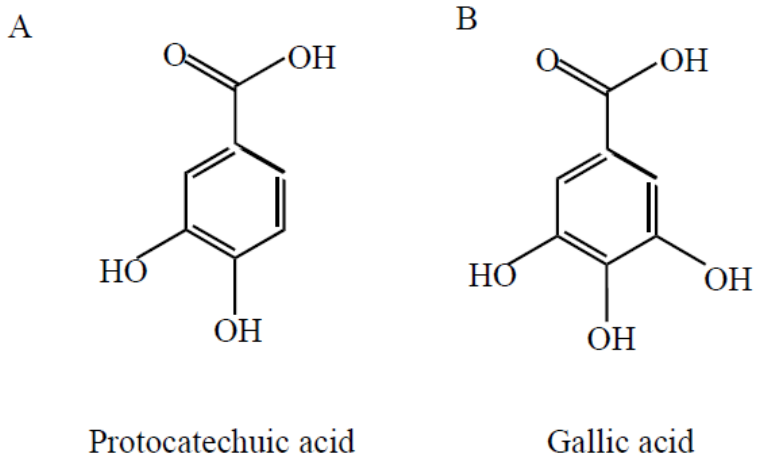


Figure 5. The chemical structure of hydroxybenzoic acids, which are (A) protocatechuic acid and (B) gallic acid.

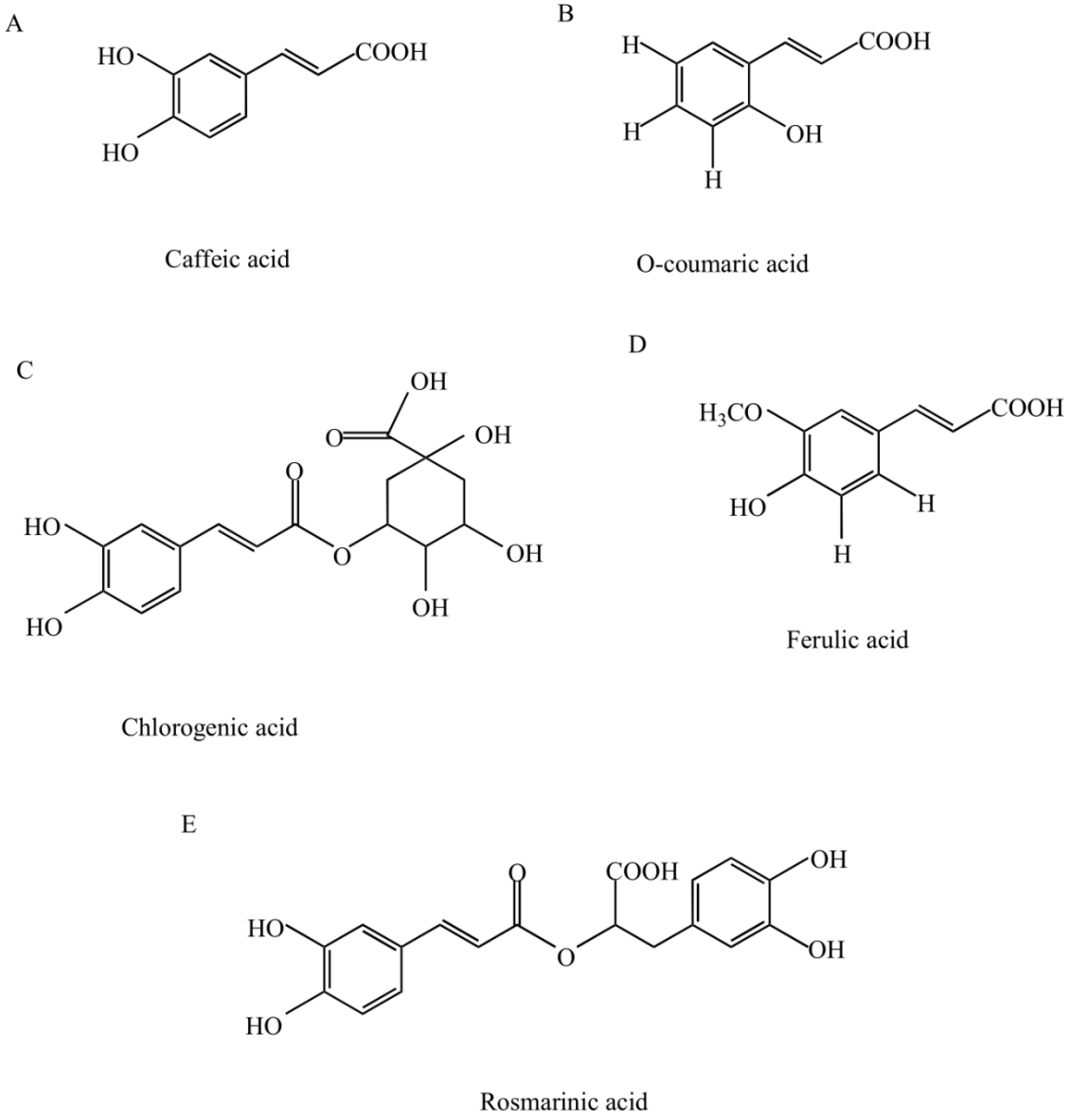
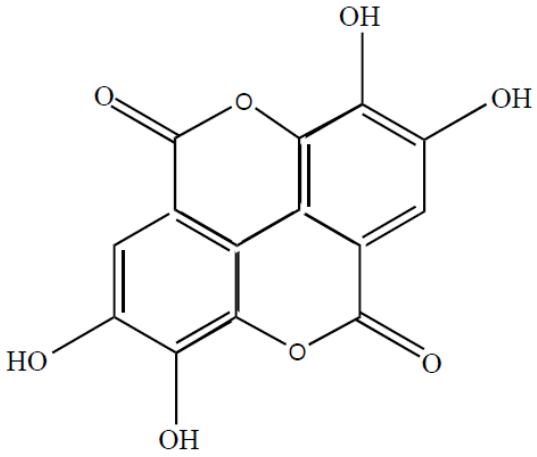


Figure 6. The chemical structure of hydroxycinnamic acids, which are (A) caffeic acid, (B) o-coumaric acid, (C) chlorogenic acid, (D) ferulic acid and (E) rosmarinic acid.

ELLAGIC ACID

Ellagic acid is a natural phenolic constituent with a molecular mass of 302.197 g/mol (Figure 7). It is an amphiphilic molecule consisting of a planar biphenyl lipophilic moiety joined by two rings and possesses four hydroxyl groups that are combined with lactone groups to form a hydrophilic moiety, which plays a crucial role in the compound's biological activity [53, 54, 55]. Currently, ellagic acid has garnered significant attention due to its remarkable antiparasitic effects [56, 57, 58].



Ellagic acid

Figure 7. The chemical structure of ellagic acid.

Antiparasitic Activities of Ellagic Acid

A group of researchers from the University of Milan made the initial discovery of ellagic acid isolated from *Tristaniopsis calobuxus* [59] and *Punica granatum* in Malaysia [60]. They found that this compound inhibited the growth of chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* in vitro [59]. Furthermore, ellagic acid's activity was found to be more pronounced during the mature stages of the parasite's lifecycle when the majority of the haemoglobin-rich host cell cytoplasm was consumed and digested [59]. One proposed mechanism of ellagic acid's antimalarial action is the suppression of beta-hematin (hemozoin) formation, a process by which the parasite neutralises the toxic by-products of haemoglobin breakdown [59]. In a recent study by Muchtar et al. (2022) [61], ellagic acid inhibited *P. falciparum* (3D7 strain) with an IC_{50} of 1.85 ± 4.57 nM. A significant change was further observed in the pH of the digestive vacuole of ellagic acid-treated parasites (pH values ranging between 6.11 to 6.74) compared to non-treated parasites (pH 5.18). Another investigation by Haeussler et al. (2019) [62] demonstrated that ellagic acid substantially inhibited *P. vivax* glucose 6-phosphate dehydrogenase (PvG6PD) with an IC_{50} of 32.5 ± 13.4 nM and *P. falciparum* glucose 6-phosphate dehydrogenase 6-phosphogluconolactonase (PfGluPho) with an IC_{50} of 77 ± 22 nM.

Ellagic acid was also reported to exhibit substantial antimalarial activity in an intraperitoneal administration study of a murine model [59]. This compound showed greater efficacy against *P. vinckei petteri* compared to artesunate. Mice treated with ellagic acid before parasite inoculation displayed a greater reduction in parasitemia than control mice, suggesting a therapeutic effect of the compound. Ellagic acid also demonstrated synergistic effects with chloroquine (FIC_{50} = 0.63 ± 0.05), mefloquine (FIC_{50} = 0.64 ± 0.09), atovaquone (FIC_{50} = 0.53 ± 0.21), and artesunate (FIC_{50} = 57 ± 0.13), resulting in lower concentrations of each drug used in combination therapy and potentially preventing drug resistance [59]. To assess its effect on chloroquine-resistant reversion in the *P. falciparum* W2 strain, ellagic acid was compared with verapamil, which is known to reverse chloroquine resistance. The study concluded that verapamil exhibited chloroquine resistance reversal while ellagic acid did not. Additionally, chloroquine-sensitive strain F32 served as a negative control in the study and did not show such reversion effect [59].

Past research also reported the antileishmanial effect of ellagic acid (maximal effect = 100 µg/mL) on *L. major* promastigote forms. Results from the MTT test revealed that

ellagic acid decreased sheep macrophage viability starting at 6.3 µg/mL, with a CC_{50} value of 23.8 µg/mL [63]. Another investigation demonstrated that ellagic acid displayed strong antileishmanial activity (IC_{50} = 151.7 µg/mL) against the promastigotes of *L. amazonensis*. Treatment with ellagic acid further resulted in a decreased percentage of amastigotes in infected macrophages [64].

Moreover, the effectiveness of ellagic acid against the cattle helminth *Haemonchus contortus* was evaluated using the adult motility and egg hatch tests in vitro. In the adult motility test, ellagic acid (concentrations ranging from 0.09-3.00 mg/mL) inhibited *H. contortus* in a concentration-dependent manner. Test worms exposed to ellagic acid at 1.5 mg/mL died six hours after exposure. In the egg hatch test, ellagic acid (concentrations ranging from 0.0125-25.00 µg/mL) demonstrated a concentration-dependent reduction in larval generation from *H. contortus eggs* [65].

In another study, rats infected with *Trypanosoma congolense* received daily ellagic acid treatments of 100 and 200 mg/kg body weight (BW) for 14 days. This compound exhibited significant trypanosuppressive activity at doses of 100 and 200 mg/kg ($p < 0.05$). Furthermore, ellagic acid considerably reduced trypanosome-induced anaemia, hepatic and renal damage, hepatomegaly, splenomegaly, and renal hypertrophy ($p < 0.05$) [66].

The mentioned IC_{50} values are promising for further in vivo testing and drug development. Analogue research and iterative medicinal chemistry have been employed to identify compounds with enhanced efficacy and pharmacological characteristics. Understanding the target molecule's structure can effectively guide medicinal chemistry efforts. Reliable, highly predictive in vitro and in vivo testing for activity, often using the same parasite species that infect humans, is advantageous for new antiparasitic drug development [67].

Lead optimisation involves designing and synthesising novel compounds using medicinal chemistry, followed by evaluating their improved properties. This iterative process impacts costs and timelines, with the lead optimisation stage being pivotal in shaping the drug discovery process [67]. Selecting a strong drug candidate at this stage involves prominent consideration of bioavailability, metabolic factors, and toxicity. It essentially involves problem-solving where the involvement of the pharmaceutical sector becomes crucial. In recent years, public-private partnerships have significantly improved the lead optimisation phase of antiparasitic drug discovery by re-engaging the pharmaceutical industry and the provision of funds. These partnerships have achieved promising results in getting drug candidates into clinical trials and addressing how businesses can contribute to the cause. Public-private partnerships have also adopted portfolio strategies that often prioritise development over discovery, despite the inherent risks in drug discovery. While government funding supports fundamental and early discovery research, collaborations between public entities in early discovery research are also viable [67].

LIMITATION OF THE NATURAL COMPOUND-BASED ANTIPARASITIC DRUG DISCOVERY

Natural product research involves numerous practical considerations, many of which become evident upon exploring the subject. These concerns encompass the reliability of indigenous medicinal data, the availability of sufficient supplies of the resource, and the potential risks of overharvesting and resource depletion [68]. Factors such as plant subspecies or age, regional and seasonal variations, extraction and storage methods, as well as climatic influences can collectively influence the quality and consistency of chemical constituents and their concentrations in natural products [69]. The isolation of pure compounds from chemically complex extracts, often used for screening, can also pose significant challenges [12]. The utilisation of local medicinal herbs may yield valuable insights into lesser-known plants; however, different communities might have diverse regional applications for the same plants. To potentially address these challenges, the establishment of smaller, specialised organizations focusing on natural product drug discovery can facilitate the licensing of their discoveries to larger entities for marketing and clinical development. An example of successful collaboration between a smaller organisation and a major pharmaceutical company is Pharmamar [71].

CONCLUSION

Plants produce an abundance of natural compounds with intriguing biological characteristics. The potential to uncover novel bioactive components with antiparasitic properties is conceivable due to the presence of natural chemicals with diverse structural variations in medicinal plants. Ellagic acid and other phenolic substances are routinely analysed in laboratories. Prior studies have demonstrated the potential antiparasitic effects of phenolic compounds, including the potential for ellagic acid to inhibit the development of parasites such as Plasmodium, Trypanosoma, Leishmania, and helminths. Further studies should be conducted to comprehensively investigate the properties of ellagic acid and other phenolic compounds.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Higher Education, Malaysia (MOHE) for providing the Fundamental Research Grant Scheme (FRGS) (FRGS/1/2019/STG03/USM/03/03). The first author also receives the Graduate Research Assistant Scheme (GRA) (2020/2021) from the FRGS. We would also like to thank the School of Health Sciences and the Universiti Sains Malaysia Libraries

(Perpustakaan Hamdan Tahir and Perpustakaan Hamzah Sendut) for providing the resources to write the review article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

1. Momčilović, S., Cantacessi, C., Arsić-Arsenijević, V., Otranto, D. and Tasić- Otašević, S. (2019) Rapid diagnosis of parasitic diseases: current scenario and future needs. *Clinical Microbiology and Infection* 25(3), 290-309.
2. Wink, M. (2012) Medicinal plants: a source of anti-parasitic secondary metabolites. *Molecules* 17(11), 12771-12791.
3. Sharma, N., Singh, V. and Shyma, K.P. (2015) Role of parasitic vaccines in integrated control of parasitic diseases in livestock. *Veterinary World* 8(5), 590-598.
4. Salmerón-Manzano, E., Garrido-Cardenas, J.A. and Manzano-Agugliaro, F. (2020) Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health* 17(10), 3376.
5. Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. and Supuran, C.T. (2021) Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery* 20, 200-216.
6. Sharifi-Rad, J., Quispe, C., Castillo, C.M.S., Caroca, R., Lazo-Vélez, M.A., Antonyak, H., Polishchuk, A., Lysiuk, R., Oliinyk, P., De Masi, L., Bontempo, P., Martorell, M., Daştan, S.D., Rigano, D., Wink, M. and Cho, W.C. (2022) Ellagic acid: a review on its natural sources, chemical stability, and therapeutic potential. *Oxidative Medicine and Cellular Longevity* 2022, 1-24.
7. Mohanty, S., Gupta, A.C., Maurya, A.K., Shanker, K., Pal, A. and Bawankule, D.U. (2021) Ameliorative effects of dietary ellagic acid against severe malaria pathogenesis by reducing cytokine storms and oxidative stress. *Frontiers in Pharmacology* 12, 777400.
8. Sepúlveda, L., Ascacio, A., Rodríguez-Herrera, R., Aguilera-Carbó, A. and Aguilar, C. (2011) Ellagic acid: Biological properties and biotechnological development for production processes. *African Journal of Biotechnology* 10(22), 4518-4523.
9. Zhang, Y., Cai, P., Cheng, G. and Zhang, Y. (2022) A brief review of phenolic compounds identified from plants: their extraction, analysis, and biological activity. *Natural Product Communications* 17(1).
10. Simões-Pires, C.A., Vargas, S., Marston, A., Ioset, J.-R., Paulo, M.Q., Matheeussen, A. and Maes, L. (2009) Ellagic acid derivatives from *Syzygium cumini* stem bark: investigation of their antiplasmodial activity. *Natural Product Communications* 4(10), 1371-1376.
11. Dardona, Z. (2023) Literature review: *Punica granatum* (pomegranate) with an emphasis on its anti-parasitic activity. *GSC Biological and Pharmaceutical Sciences* 23(2), 100-114.
12. Tagboto, S. and Townson, S. (2001) Antiparasitic properties of medicinal plants and other naturally occurring products. *Advances in Parasitology* 50, 199-295.
13. Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, P., Frappier, F., Laurens, A. and Hocquemiller, R. (2004) Antiparasitic activities of medicinal plants used in Ivory Coast. *Journal of Ethnopharmacology* 90(1), 91-97.
14. Ali, R., Tabrez, S., Rahman, F., Alouffi, A.S., Alshehri, B.M., Alshammari, F.A., Alaidarous, M.A., Banawas, S., Dukhyil, A.A.B. and Rub, A. (2021) Antileishmanial evaluation of bark methanolic extract of *Acacia nilotica*: *In vitro* and in silico Studies. *ACS Omega* 6(12), 8548-8560.
15. Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. and Chen, S. (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* 21(10), 1374.
16. Minatel, I.O., Borges, C.V., Ferreira, M.I., Gomez, H.A.G. and Lima, C.-Y.O.C. and G.P.P. (2017) Phenolic compounds: functional properties, impact of processing and bioavailability, phenolic compounds - biological activity. *IntechOpen.* DOI: 10.5772/66368.
17. Pandey, K.B. and Rizvi, S.I. (2009) Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity* 2(5), 270-278.
18. Albuquerque, B.R., Heleno, S.A., Oliveira, M.B.P.P., Barros, L. and Ferreira, I.C.F.R. (2021) Phenolic compounds: current industrial applications, limitations and future challenges. *Food & Function* 12(1), 14-29.
19. Panche, A.N., Diwan, A.D. and Chandra, S.R. (2016) Flavonoids: an overview. *Journal of Nutritional Science* 5, e47.
20. Mead, J.R. and McNair, N. (2006) Antiparasitic activity of flavonoids and isoflavones against *Cryptosporidium parvumand Encephalitozoon intestinalis*. *FEMS Microbiology Letters* 259(1), 153-157.
21. Forney, J.R., DeWald, D.B., Yang, S., Speer, C.A. and Healey, M.C. (1999) A role for host phosphoinositide 3-kinase and cytoskeletal remodeling during *Cryptosporidium parvum* infection. *Infection and Immunity* 67(2), 844-852.
22. MacLaren, A., Attias, M. and de Souza, W. (2004) Aspects of the early moments of interaction between tachyzoites of *Toxoplasma gondii* with neutrophils. *Veterinary Parasitology* 125(3-4), 301-312.
23. Kim, H., Hong, S.-H., Jeong, H.E., Han, S., Ahn, J., Kim, J.-A., Yang, J.-H., Oh, H.J., Chung, S. and Lee, S.-E. (2022) Microfluidic model for in vitro acute *Toxoplasma gondii* infection and transendothelial migration. *Scientific Reports* 12(1), 11449.
24. Rudrapal, M. and Chetia, D. (2016) Plant flavonoids as potential source of future antimalarial leads. *Systematic Reviews in Pharmacy* 8(1), 13-18.
25. Lim, S.S., Kim, H.S. and Lee, D.U. (2007) In vitro antimalarial activity of flavonoids and chalcones. *Bulletin Korean Chemistry Society* 28(12), 2495-2497.
26. Rasoanaivo, P., Wright, C.W., Willcox, M.L. and Gilbert, B. (2011) Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria Journal* 10 Suppl 1(Suppl 1), S4.
27. Ntie-Kang, F., Onguéné P.A., Lifongo, L.L. and Luc, M.M. (2014) The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal* 13(1), 81.
28. Araújo, M.V., Queiroz, A.C., Silva, J.F.M., Silva, A.E., Silva, J.K.S., Silva, G.R., Silva, E.C.O., Souza, S.T., Fonseca, E.J.S., Camara, C.A., Silva, T.M.S. and Alexandre-Moreira, M.S. (2019) Flavonoids induce cell death in *Leishmania amazonensis*: in vitro characterization by flow cytometry and Raman spectroscopy. *The Analyst* 144(17), 5232–5244.
29. Phumee, A., Jariyapan, N., Chusri, S., Hortiwakul, T., Gay, F., Limpanasithikul, W. and Siriyasatien, P. (2020) Determination of anti-

leishmanial drugs efficacy against *Leishmania martiniquensis* using a colorimetric assay. *Parasite Epidemiology and Control* 9(00143).

30. Ginouvès, M., Simon, S., Nacher, M., Demar, M., Carme, B., Couppié, P. and Prévot, G. (2017) *In vitro* sensitivity of cutaneous *Leishmania promastigote* isolates circulating in French Guiana to a set of drugs. *The American Journal of Tropical Medicine and Hygiene* 96(5), 1143-1150.

31. Mehwish, S., Varikuti, S., Ali Khan, M., Khan, T., Khan, I.U., Satoskar, A., Elsayed Elserehy, H.A. and Ullah, N. (2021) Bioflavonoid-induced apoptosis and DNA damage in amastigotes and promastigotes of *Leishmania donovani*: deciphering the mode of action. *Molecules* 26(19), 5843.

32. Razak, R.A., Suzery, M., Razali, R., Amin, Z., Mokhtar, R.A.M., Lee, P.C. and Budiman, C. (2021) Technical data on the inhibition properties of some medicinal plant extracts towards caseinolytic protease proteolytic subunit of *Plasmodium knowlesi*. *Data in Brief* 39**,** 107588.

33. Adamczyk, B., Simon, J., Kitunen, V., Adamczyk, S. and Smolander, A. (2017) Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. *ChemistryOpen,* 6(5), 610-614.

34. Izumi, T. and Terauchi, M. (2020) The diverse efficacy of food-derived proanthocyanidins for middle-aged and elderly women. *Nutrients* 12(12), 3833.

35. Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. and Hoskin, S.O. (2006) The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* 22(6), 253-261.

36. Lutgen, P. (2018) Tannins in Artemisia: the hidden treasure of prophylaxis. *Pharmacy & Pharmacology International Journal* 6(3).

37. Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I. and Thamsborg, S.M. (2014) Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PloS one* 9(5), e97053.

38. Li, Y., Xie, S., Ying, J., Wei, W. and Gao, K. (2018) Chemical structures of lignans and neolignans isolated from Lauraceae. *Molecules* 23(12), 3164.

39. da Silva Filho, A.A., Costa, E.S., Cunha, W.R., e Silva, M.L., Nanayakkara, N.P. and Bastos, J.K. (2008) In vitro antileishmanial and antimalarial activities of tetrahydrofuran lignans isolated from *Nectandra megapotamica* (Lauraceae). *Phytotherapy Research* 22(10), 1307-1310.

40. Sartorelli, P., Salomone Carvalho, C., Quero Reimão, J., Lorenzi, H. and Tempone, A. (2010) Antitrypanosomal activity of a diterpene and lignans isolated from *Aristolochia cymbifera*. *Planta Medica* 76(13), 1454-1456.

41. Raiguru, B.P., Mohapatra, S., Nayak, S., Sahal, D., Yadav, M. and Acharya, B.N. (2022) Flavone-stilbene hybrids: synthesis and evaluation as potential antimalarial agents. *European Journal of Medicinal Chemistry Reports* 4, 100029.

42. Bruno, F., Castelli, G., Vitale, F., Catanzaro, S., Badaco, V.V., Roberti, M., Colomba, C., Cascio, A. and Tolomeo, M. (2021) Antiparasitic effect of stilbene and terphenyl compounds against *Trypanosoma cruzi* parasites. *Pharmaceuticals (Basel, Switzerland)* 14(11), 1199.

43. Gani, M.A. and M, S. (2021) Phenolic compounds. Bioactive compounds - biosynthesis, characterization and applications. *IntechOpen*. DOI: 10.5772/intechopen.91131.

44. Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B. and Uddin, M.S. (2021) Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules* 27(1), 233.

45. Kumar, N. and Goel, N. (2019) Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports* 24, e00370.

46. Kaurinovic, B. and Vastag, D. (2019) Flavonoids and phenolic acids as potential natural antioxidants. Antioxidants. *IntechOpen*. DOI: 10.5772/intechopen.83731.

47. Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.-H. and Jaremko, M. (2020) Important flavonoids and their role as a therapeutic agent. *Molecules* 25(22), 5243.

48. Kaushik, B., Sharma, J., Yadav, K., Kumar, P. and Shourie, A. (2021) Phytochemical properties and pharmacological role of plants: secondary metabolites. *Biosciences Biotechnology Research Asia* 18(1), 23-35.

49. Fordjour, P.A., Adjimani, J.P., Asare, B., Duah-Quashie, N.O. and Quashie, N.B. (2020) Antimalarial activity of phenolic acids is structurally related. *Research Square,* https://doi.org/10.21203/rs.3.rs-21702/v1

50. Antwi, C.A., Amisigo, C.M., Adjimani, J.P. and Gwira, T.M. (2019) In vitro activity and mode of action of phenolic compounds on *Leishmania donovani*. *PLOS Neglected Tropical Diseases* 13(2), e0007206.

51. Patel, M., Ganeshpurkar, A., Bansal, D. and Dubey, N. (2015) Experimental evaluation of anthelmintic effect of gallic acid. *Pharmacognosy Communications* 5(2), 145-147.

52. Amisigo, C.M., Antwi, C.A., Adjimani, J.P. and Gwira, T.M. (2019) In vitro anti-trypanosomal effects of selected phenolic acids on *Trypanosoma brucei*. *PLOS one,* 14(5), e0216078.

53. Zuccari, G., Baldassari, S., Ailuno, G., Turrini, F., Alfei, S. and Caviglioli, G. (2020) Formulation strategies to improve oral bioavailability of ellagic acid. *Applied Sciences,* 10(10), 3353.

54. Lee, M.R. (2018) Entrapment of ellagic acid in dairy protein-based nanoparticles. *Journal of Milk Science and Biotechnology* 36(2), 121-124.

55. Zulhendri, F., Chandrasekaran, K., Kowacz, M., Ravalia, M., Kripal, K., Fearnley, J. and Perera, C.O. (2021) Antiviral, antibacterial, antifungal, and antiparasitic properties of propolis: a Review. *Foods* 10(6), 1360.

56. Beshbishy A.M., Batiha G.E., Yokoyama N., Igarashi I. (2019) Ellagic acid microspheres restrict the growth of *Babesia* and *Theileria* in vitro and *Babesia microti* in vivo. *Parasites & Vectors* 12(1), 269.

57. Nik Mat Zin, N.N.I., Ibrahim, N., Zakaria, Y. and Abu Bakar, N(2021). The acetone crude extract of *Quercus infectoria* (Olivier) galls alters pH of the digestive vacuole of the malaria parasite, *Plasmodium falciparum*. *Tropical Biomedicine* 38(2), 40-47.

58. Cuijie, G., Changjiang, Z., Xinxin, H., Jiahao, L., Linjiao, H.E., Fan, Y., Fei'er, R. and Kaixu, C. (2022). Effect of supplementary ellagic acid on parasitic infection in lactating Foals. *China Animal Husbandry and Veterinary Medicine* 49(12), 4936-4944.

59. Soh, P.N., Witkowski, B., Olagnier, D., Nicolau, M.-L., Garcia-Alvarez, M.-C., Berry, A. and Benoit-VicalF. (2009) In vitro and in vivo properties of ellagic acid in malaria treatment. *Antimicrobial Agents and Chemotherapy* 53(3), 1100-1106.

60. BenSaad, L.A., Kim, K.H., Quah, C.C., Kim, W.R. and Shahimi, M. (2017) Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from *Punica granatum*. *BMC Complementary and Alternative Medicine* 17(1), 47

61. Muchtar, N.H., Nik Mat Zin, N.N.I., Mohamad, F.S. and Abu-Bakar, N. (2022) Ellagic acid induces in vitro alkalinisation of the digestive vacuole in drug-sensitive *Plasmodium falciparum* Strain. *Malaysian Journal of Medical Sciences* 29(4), 43-52.

62. Haeussler, K., Berneburg, I., Jortzik, E., Hahn, J., Rahbari, M., Schulz, N., Preuss, J., Zapol'skii, V.A., Bode, L., Pinkerton, A.B., Kaufmann, D.E., Rahlfs, S. and Becker, K, (2019) Glucose 6-phosphate dehydrogenase 6-phosphogluconolactonase: characterization of the *Plasmodium vivax* enzyme and inhibitor studies. *Malaria Journal* 18(2).

63. Alves, M.M.M., Brito, L.M., Souza, A.C., Queiroz, B.C.S.H., de Carvalho, T.P., Batista, J.F., Oliveira, J.S.S.M., de Mendonça, I.L., Lira, S.R.S., Chaves, M.H., Gonçalves, J.C.R., Carneiro, S.M.P., Arcanjo, D.D.R. and Carvalho, F.A.A. (2017) Gallic and ellagic acids: two natural immunomodulator compounds solve infection of macrophages by *Leishmania major. Naunyn-Schmiedeberg's Archives of Pharmacology* 390(9), 893-903.

64. Dutra, R.P., Bezerra, J.L., Silva, M.C.P. da, Batista, M.C.A., Patrício, F.J.B., Nascimento, F.R.F., Ribeiro, M.N.S. and Guerra, R.N.M. (2019) Antileishmanial activity and chemical composition from Brazilian geopropolis produced by stingless bee *Melipona fasciculata*. *Revista Brasileira de Farmacognosia* 29(3), 287-293.

65. Mondal, H., Hossain, H., Awang, K., Saha, S., Islam, K., Rahman, M., Jahan, I., Rahman, M. and Shilpi, J. (2014) Anthelmintic activity of ellagic acid, a major constituent of *Alternanthera sessilis* against *Haemonchus contortus*. *Pakistan Veterinary Journal* 35(1), 58-62.

66. Aminu, R., Ibrahim, M.A., Rahman, M.A., Dash, R. and Umar, I.A. (2017) Trypanosuppresive effects of ellagic acid and amelioration of the trypanosome-associated pathological features coupled with inhibitory effects on trypanosomal sialidase in vitro and in silico. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 30, 67-73.

67. Pink, R., Hudson, A., Mouriès, M.-A. and Bendig, M. (2005) Opportunities and challenges in antiparasitic drug discovery. *Nature Reviews Drug Discovery* 4(9), 727-740.

68. Howes, M.R., Quave, C.L., Collemare, J., Tatsis, E.C., Twilley, D., Lulekal, E., Farlow, A., Li, L., Cazar, M., Leaman, D.J., Prescott, T.A.K., Milliken, W., Martin, C., De Canha, M.N., Lall, N., Qin, H., Walker, B.E., Vásquez-Londoño, C., Allkin, B. and Rivers, M. (2020) Molecules from nature: reconciling biodiversity conservation and global healthcare imperatives for sustainable use of medicinal plants and fungi. *Plants, People, Planet* 2(5), 463-481.

69. Yun, B.-W., Yan, Z., Amir, R., Hong, S., Jin, Y.-W., Lee, E.-K. and Loake, G.J (2012) Plant natural products: history, limitations and the potential of cambial meristematic cells. *Biotechnology and Genetic Engineering Reviews* 28(1), 47-60.

70. Jayawardene, K.L.T.D., Palombo, E.A. and Boag, P.R. (2021) Natural products are a promising source for anthelmintic drug discovery. *Biomolecules* 11(10), 1457.

71. Beutler, J.A. (2019) Natural Products as a Foundation for Drug Discovery. *Current Protocols in Pharmacology* 86(1).