A systematic review of pentacyclic triterpenes and their derivatives as chemotherapeutic agents against tropical parasitic diseases

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SUMMARY

Parasitic infections are among the leading global public health problems with very high economic and mortality burdens. Unfortunately, the available treatment drugs are beset with side effects and continuous parasite drug resistance is being reported. However, new findings reveal more promising compounds especially of plant origin. Among the promising leads are the pentacyclic triterpenes (PTs) made up of the oleanane, ursane, taraxastane, lupane and hopane types. This paper reviews the literature published from 1985 to date on the *in vitro* and *in vivo* anti-parasitic potency of this class of phytochemicals. Of the 191 natural and synthetic PT reported, 85 have shown high anti-parasitic activity against various species belonging to the genera of *Plasmodium, Leishmania, Trypanosoma*, as well as various genera of Nematoda. Moreover, structural modification especially at carbon 3 (C3) and C27 of the parent backbone of PT has led to improved anti-parasitic activity in some cases and loss of activity in others. The potential of this group of compounds as future alternatives in the treatment of parasitic diseases is discussed. It is hoped that the information presented herein will contribute to the full exploration of this promising group of compounds as possible drugs for parasitic diseases.

Key words: Pentacyclic triterpenes, anti-parasitic, *Plasmodium*, *Leishmania*, *Trypanosoma*, Nematoda.

INTRODUCTION

Tropical parasitic diseases have been a serious public health problem especially in middle- and low-income countries. These diseases which include malaria, trypanosomiasis, leishmaniasis, schistosomiasis, lymphatic filariasis and onchorcerciasis affect millions of people, resulting in thousands of death annually. The disability-adjusted life year lost estimate for these diseases is very high with a combined annual magnitude of more than 70 million by 2011 (Bhutta *et al*. 2014; Hotez *et al*. 2014). At present, there have been reports on the spread of parasitic infections in non-endemic areas which raised more concerns about the feasibility of the global control strategy (Leder *et al*. 2013). The main obstacles in the control of parasitic diseases are the drugs resistance, toxicity and non-affordability of the available drugs (Buckner *et al*. 2012). This has prompted a continuous search for safer and more effective treatments especially from natural sources. In this regard, plants have been a prime target for novel therapeutic agents as evident from the large volume of studies being conducted on medicinal plants documented in scientific databases (Rocha *et al*. 2005; Wright, 2010; Izumi *et al*. 2011; Ibrahim *et al*. 2014). Interestingly, considerable success has been recorded with about 65% of all anti-parasitic agents marketed from 1981 to 2010 being originally derived from plant sources and sometimes with synthetic modifications (Newman and Cragg, 2012). This further stimulates research activities on this important area to identify novel bioactive anti-parasitic agents that could potentially be used to combat tropical parasitic diseases. Fortunately, a number of bioactive agents, such as flavonoids, curcuminoids and triterpenoids have shown promising anti-parasitic activities that warrant further drug development studies (Rasoanaivo *et al*. 2011; Ibrahim *et al*. 2014).

Pentacyclic triterpenes (PTs) belong to a group of widespread isoprene-derived secondary metabolites

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collectively known as triterpenes (a sub-class of terpenes). PTs are synthesized mainly by the cyclization of oxidosqualene and squalene and exist in their free form or as components of saponins (glycosides) in many tropical and subtropical plants (Xu *et al*. 2004; Jäger *et al*. 2009). The compounds have attracted attention due to their remarkable biological activities. With regard to this, three groups of PT, namely; the oleanane, ursane and lupane groups are considered to be the most important (Dzubak *et al*. 2006), although other groups such as hopane, taraxastane and friedelane types may also be important. Thus, various derivatives of the biologically important groups of PT are synthesized with the aim of lowering the toxicity and/or increasing the therapeutic activity of the parent compounds. Some of these PT are already registered and/or being marketed in some parts of the world as clinical drugs for the treatment of liver related diseases and diabetes, while others are at various phases of clinical trials (Sheng and Sun, 2011).

Presently, update on the newly discovered PT is a subject of annual review, suggesting an interest to keep track of the advances made in the study of this group of compounds (Dzubak *et al*. 2006). Moreover, various biological activities, chemistry and therapeutic potencies of the group have been reviewed to highlight the full potencies of this group of compounds. Among the available reviews are the chemistry and metabolic disease-ameliorative effects (Sheng and Sun, 2011), anti-cancer (Laszczyk, 2009), anti-inflammatory (Safayhi and Sailer, 1997), anti-microbial (Wolska *et al*. 2010), anti-chagasic (da Silva Ferreira *et al*. 2013*b*) and other pharmacological activities (Dzubak *et al*. 2006). However, a compiled review focusing on the activities of PT against broad range of parasites is lacking. This is despite the potent activities of various members of the group against different parasites as well as the crucial need to develop novel anti-parasitic agents. Hence, a review focusing on the anti-parasitic properties of PT will serve as complementary information in the spectrum of the biological activities of this promising group of phytochemicals.

Available data on plant derived PT investigated for activities against the tropical parasitic infections are reviewed in this paper. This will serve as up-to-date information that could provide direction for future scientific research as well as the future application of this group of compounds as anti-parasitic agents. The article could contribute to the search for effective drugs, which is fundamental in the global fight against parasitic infections.

METHODS

The information presented here is based on PubMed, Medline, SciFinder and Google Scholar search for the PT and their derivatives reported to be tested against parasites of the genera *Trypanosoma, Plasmodium, Leishmania, Schistosoma* and others, which are considered of importance to tropical countries. Some articles were found through tracking citations in other publications or by accessing the journals' websites. Various keywords were permutated for the search which include: PT, oleanane, friedelane, ursane, taraxasterane, lupane, hopanes, saponins, anti-parasitic, anti-plasmodial, anti-leishmanial, anti-trypanosomal, anti-filarial, nematicidal and schistomicidal. To the best of our knowledge, all the articles that reported a plant-derived pentacyclic triterpenoid and nortriterpenoids tested against a parasite were included in this paper. Other articles that reported on synthetic modifications of the plant-derived PT were also included to enable full discussion on the structure-activity relationship. In cases where an article contains the name of a compound only, the structures used in this article were obtained from publication series by Hill and Connolly (2015). On the other hand, the names provided in the articles that used nuclear magnetic resonance spectroscopy (NMR) data in validating the structure of the compounds and synthetic compounds are used. Plant names and families were verified at http://www.theplant list.org database. Overall, the information obtained covered the period; 1985 to the date of submission of this paper.

RESULTS AND DISCUSSION

A total of 112 naturally occurring PT and saponins isolated from 69 plants belonging to 35 families are reported in this paper. Ten of the total number of the compounds are nortriterpenoids of the quinone methide (possessing friedo-oleanane structure) type isolated mainly from five species of the Celastraceae family. Moreover, 62 of the total number of the compounds are the oleanane (including -friedelanes and -saponins), 19 ursane (-saponins), five taraxastane, 15 lupane (-saponins) and one hopane types of PT. These were isolated mostly from the Araliceae, Rubiaceae, Melastomataceae, Compositae and Lamiaceae plant families. Alongside these naturally occurring PT, 79 synthetic PT were also reported, of which 15 are oleanane types, nine are ursane types, one taraxastane type and 54 are lupane types. The structures of all the compounds are provided in Supplementary Fig. 1 (available from http://journals.cambridge.org/PAR).

The anti-parasitic activities of all the PTs were classified as high, moderate or low/no using the criteria suggested by Pink *et al*. (2005) and Bero *et al*. (2011) with modifications. Compounds with high potency (*in vitro* $IC_{50} \leqslant 10\ \mu g\ mL^{-1}$ against protozoa,), moderate potency (*in vitro* IC_{50} 10–20 $\mu g\ mL^{-1}$ against protozoa) and low/no activities ($IC_{50} > 20\ \mu g\ mL^{-1}$ against protozoa) are summarized in Supplementary

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Tables S1, S2 and S3, respectively (available from http://journals.cambridge.org/PAR). Activity of the compounds against other parasites beside protozoa is classified based on the dosage and activity of the standard drug used in the respective studies. Some compounds were tested in *in vivo* assays which are also summarized in Table 1.

In order to provide a clear view on the anti-parasitic potential of the PT, logical discussions on the activities of the PT against various parasites are made under separate subheadings. The investigated parasites were found to be different species of *Plasmodium, Leishmanium* and *Trypanosoma*, as well as various nematodes and *Toxoplasma gondii*. Finally, some safety and toxicity profiles of the compounds are briefly discussed.

Brief chemistry of PTs

As shown in Fig. 1A–E, the PTs of the quinone methides, oleanane and ursane groups generally have five fused six-membered rings (designated a–e), while the lupane and hopane types have four six-membered and one five-membered rings. The distinguishing feature between the oleanane and ursane types is the localization of the methyl group on the 'e' ring, whereas the taraxasteranes differ in the orientation of substituents and the positions of double bonds in the backbone. Also, the lupane and the hopane skeletons differ on the localization of the isopropenyl group on the 'e' ring. In plants, all these groups of PT (except the nortriterpenoids quinone methides) commonly originate from cyclization of squalene and oxidosqualene via multiple enzymatic and redox stages involving formation of carbocations (Xu *et al*. 2004; Vincken *et al*. 2007). Moreover, in the PT possessing the oleanane and ursane backbone, the C4, C17 and C20 appear to show the highest diversity and unsaturation as well as formation of epoxides, whilst oxygen bridges are formed between the various carbon atoms (Vincken *et al*. 2007). On the other hand, the C3 and the substituent at C17 have been the primary target for synthetic modification. Finally, the saponins of the various PT are formed via attachment of diverse sugar subunits (ranging from 1 to 8 subunits) to the parent skeleton especially at C3 and C17 and rarely on C4, C16, C20, C21 and C22 (Vincken *et al*. 2007). Although the physico-chemical properties of saponins as well as the non-glycosylated PT are as diverse as the compounds themselves, the sugar moiety on saponins tend to make them more soluble than the corresponding aglycone (Güçlü-Üstündağ and Mazza, 2007).

Anti-plasmodial activities of PTs

Perhaps the most *in vitro* active anti-plasmodial plant derived PT belong to the small group of quinone methides. Almost all the compounds belonging to the group isolated from different sources were highly active against both chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum*. The compounds are pristimerin (***1***), isoiguesterol (***2***), celastrol (***3***), 28-hydroxyisoiguesterin (***4***), 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (***5***), 28-*nor*-isoiguesterin-17-carbaldehyde (***6***) and Tingenin B (***7***) which all possessed very low IC_{50} values (<0·5 $\mu g\ mL^{-1}$) against *P. falciparum* (Supplementary Table S1, available from http://journals.cambridge.org/PAR) (Figueiredo *et al*. 1998; Maregesi *et al*. 2010; Ruphin *et al*. 2013).

The oleanane PT also showed high to low activity against *Plasmodia*. Epi-Oleanolic acid (OA) (***11***) from *Viola verecunda* inhibited the growth of the chloroquine sensitive D10 strain of *P. falciparum* with a very low IC_{50} of 0·018 $\mu g\ mL^{-1}$ which was close to that of artemisinin (0·015 $\mu g\ mL^{-1}$) (Moon *et al*. 2007). However, the same compound isolated from *Celaenodendron mexicanum* had moderate activity against multidrug resistant K1 strain of the parasite (IC_{50} 12·92 $\mu g\ mL^{-1}$) (Camacho *et al*. 2000). Another oleanane PT with potent anti-*P. falciparum* activities is 1-O-[α-L-(rhamnopyranosyl)]-23-acetoimberbic acid 29-methyl ester (***12***) from *Pittosporum mannii* (IC_{50} 1·2 $\mu g\ mL^{-1}$) (Nyongbela *et al*. 2013). Furthermore, OA (***13***) isolated from different plant species has been shown to possess anti-plasmodial activities with IC_{50} ranging from 2·1 $\mu g\ mL^{-1}$ against chloroquine sensitive clone D6 (He *et al*. 2005) to 88 $\mu g\ mL^{-1}$ against multidrug resistant K1 strain of *P. falciparum* (Steele *et al*. 1999). Large variation in IC_{50} values for the same compound often reflects the differences in the parasite strain or sometimes different experimental procedures. Moreover, the variations in the documented anti-plasmodial activities of OA might suggest that strain differences are critical for the anti-plasmodial effects of the oleananes.

Another moderately active anti-plasmodial oleanane PT and the most extensively investigated is maslinic acid (MA) (***61***). Incubation of different concentrations of the compound obtained from the fruits of *Olea europaea* with *P. falciparum* (at different growth stages) showed that the compound arrests the maturation of the intraerythrocytic parasites from early-ring to schizont stages. The IC_{50} for the chloroquine sensitive and chloroquine resistant strains of the parasite were 15·13 and 12·29 $\mu g\ mL^{-1}$, respectively (Moneriz *et al*. 2011*a*). The proposed mechanism of the anti-plasmodial activity of oleanane-type PT is via incorporation into the erythrocytes membrane thereby modifying accessibility of the parasites into the cells (Sairafianpour *et al*. 2003). Indeed, other studies have demonstrated that PT exert their anti-parasitic activities via an interaction with the host cell membranes (Ziegler *et al*. 2006; Broniatowski *et al*. 2012).

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Table 1. *In vivo* antiparasitic activities of pentacyclic triterpenes

Class	Compound	Plant	Parasites/dosage used	Activity	Reference
Oleanane; Quinone methide	(***5***)	*Salacia kraussii* (Harv.) Harv. (Celastraceae)	*P. berghei* (10 mg kg^{-1} bw)	Inactive	Figueiredo *et al.* (1998)
	(***23–28***)	*Maesa balansae* Mez (Myrsinaceae)	*L. infantum:* amastigotes (0·2–0·4 mg kg^{-1} bw)	>90% parasite reduction after 1-week treatment	Germonprez *et al.* (2005)
	(***25***)	*Maesa balansae* Mez (Myrsinaceae)	*L. donovani* amastigotes: (0·2–0·8 mg kg^{-1} bw)	>90% parasite reduction after 1-week treatment	Maes *et al.* (2004)
	(***61***)	–	*P. yoelii* (40 mg kg^{-1} bw)	>80% survival rate after 1-week treatment	Moneriz *et al.* (2011*b*)
	(***13***)	*Miconia fallax* DC (Melastomataceae)	*T. cruzi* (50 mg kg^{-1} day^{-1})	76% parasite reduction after 1-week treatment	da Silva Ferreira *et al.* (2013*a*)
		–	*T. cruzi* (20 mg kg^{-1} day^{-1} oral)	40% parasite reduction after 3-week treatment	da Silva Ferreira *et al.* (2010)
		Lantana camara L. (Verbenaceae)	*B. malayi* (200 mg kg^{-1} bw oral and 100 mg kg^{-1} bw intraperitoneal)	Inactive against microfilariid; 18·18% macrofilacidal activity compared to untreated control for 5 days	Misra *et al.* (2007)
	(***66***)	*Lantana camara* L. (Verbenaceae)	*B. malayi* (200 mg kg^{-1} bw oral and 100 mg kg^{-1} bw intraperitoneal)	Inactive against microfilariid and macrofilariid after 5 days treatment	Misra *et al.* (2007)
	(***33***)	–	*L. donovani* promastigotes (50 mg kg^{-1} bw day^{-1} 3 times 5 day apart)	100% parasite clearance by the end of 45 days	Ukil *et al.* (2005)
	(***36***, ***37***)	–	*B. malayi* (100 mg kg^{-1})	54% parasite death after 5 days treatment	Kalani *et al.* (2013)
Ursane	(***89***)	*Mimusops caffra* E. Mey. ex A.DC (Sapotaceae)	*P. berghei*	94·01% parasite reduction after 4-day treatment	Simelane *et al.* (2013)
	(***88***)	–	*T. cruzi* (20 mg kg^{-1} day^{-1} oral)	60% parasite reduction after 3-week treatment	da Silva Ferreira *et al.* (2010)
		Miconia fallax DC (Melastomataceae)	*T. cruzi* (50 mg kg^{-1} day^{-1})	79% parasite reduction after 1-week treatment	da Silva Ferreira *et al.* (2013*a*)
		Miconia sellowiana Naud. (Melastomataceae)	*T. cruzi* (2 mg kg^{-1} bw day^{-1})	75·7% parasite reduction after 1-week treatment	Cunha *et al.* (2006)
	(***93***)	–	*T. cruzi* (2 mg kg^{-1} bw day^{-1})	70·4% parasite reduction after 1-week treatment	Cunha *et al.* (2006)
Taraxastane	(***115***)	*Pluchea lanceolata* DC. (Asteraceae)	*P. berghei* (10 mg kg^{-1} bw)	51·20% suppression of parasitaemia after 1-week treatment	Mohanty *et al.* (2013)
Lupane	(***140***)	*Bacopa monniera* Hayata & Matsum. (Plantaginaceae)	*L. donovani* (10 mg kg^{-1} bw)	92% parasite reduction after 6-week treatment	Chowdhury *et al.* (2003)
	(***130***)	–	*P. berghei* (100 mg kg^{-1} bw)	70% parasite reduction after 7 days treatment	De Sá *et al.* (2009)
	(***129***)	*Uapaca nitida* Müll-Arg. (Euphorbiaceæ)	*P. berghei* (0–250 mg kg^{-1} day^{-1})	Inactive	Steele *et al.* (1999)
	(***128***)	*Vernonia Brasiliana*	*P. berghei* (15 mg kg^{-1} bw)	Inactive	Alves *et al.* (1997)

On the other hand, some oleanane PT such as *β*-amyrin (***19***), arjun glucoside (***73***), sericoside (***74***), and maytensifolin B (***22***) were shown to possess very low or no anti-plasmodial activity (Supplementary Table S3, available from http://journals.cambridge.org/PAR). However, it is also noteworthy that these low active anti-plasmodial oleanane PT totally lack an acid group and/or the C3 hydroxyl or these groups are derivatized/sterically hindered (Cunha *et al.* 2003). This signifies the role of the polar groups at C27 and C3 in the anti-plasmodial activity of this class of PT.

In the ursane group, ursolic acid (UA) (***88***) isolated from *Baccharis dracunculifolia* had the highest reported *in vitro* activity against chloroquine sensitive *P. falciparum* with IC_{50} of 1 $\mu g\ mL^{-1}$ (da Silva Filho

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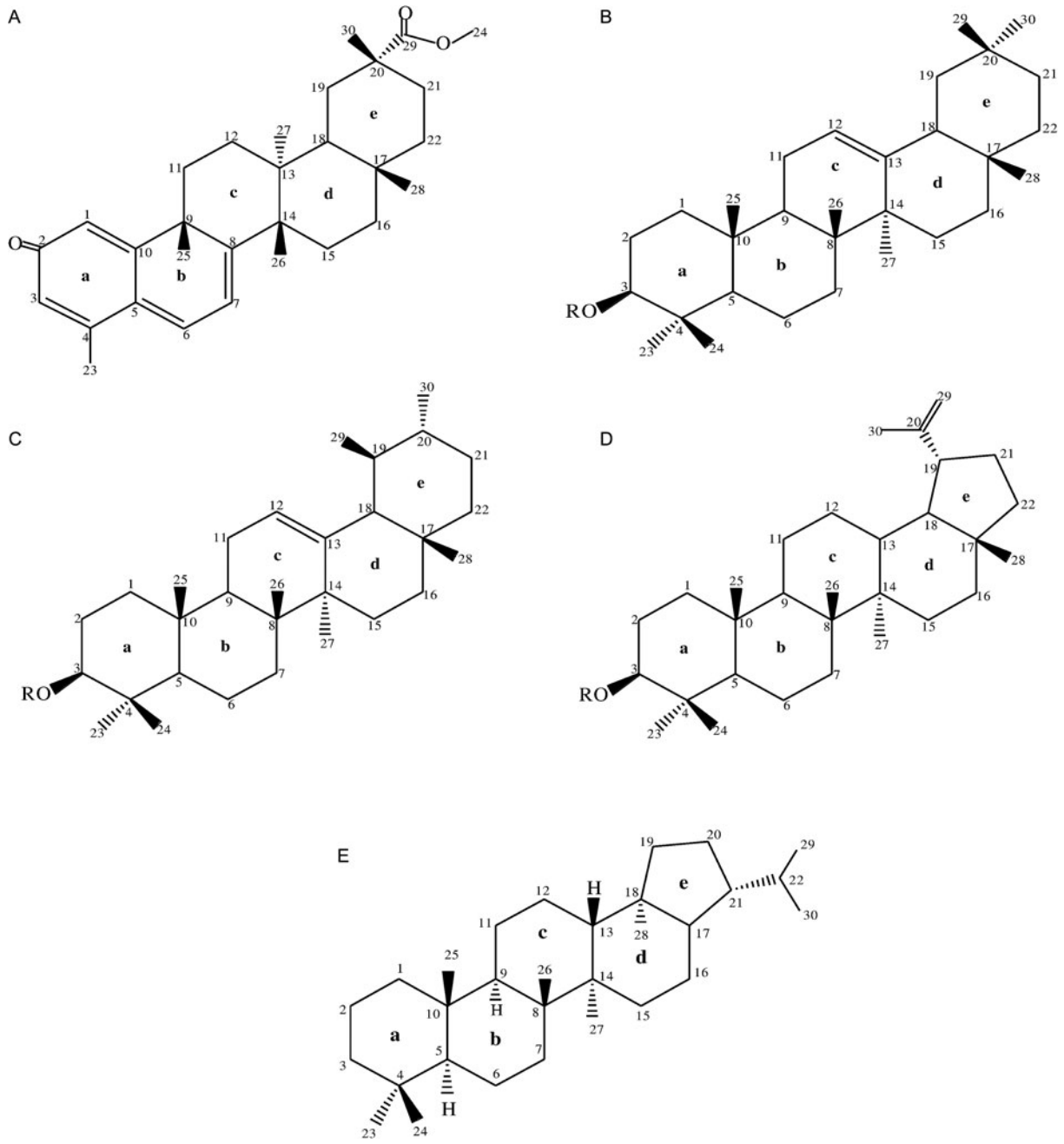

Fig. 1. Representative skeletons of the different classes of pentacyclic triterpenes showing the carbon numbers and ring annotations. (A) Quinone methides, (B) oleananes, (C) ursanes, (D) lupanes, and (E) taraxastanes.

et al. 2009). Additionally, UA from the leaves of *Mimusops caffra* showed an IC_{50} of 6·8 $\mu g\,mL^{-1}$ against chloroquine sensitive D10 strain of *P. falciparum* (Simelane *et al*. 2013). The activity was boosted by derivatization of the compound to 3β-O-acetylursolic acid (***89***) and 3-oxo-ursolic acid (***99***) with IC_{50} of 1·9 and 7·3 $\mu g\,mL^{-1}$ respectively, using the same organism. However, other reports on the anti-plasmodial activity of UA contradict the above findings. For instance, Suksamrarn *et al*. (2003) and Graziose *et al*. (2012) reported UA to be inactive against multidrug resistant and chloroquine sensitive strains of *P. falciparum* respectively. It is pertinent to note that the authors used either different methods or compound dilutions in the anti-plasmodial assay protocol which highlights the need for harmonization of protocols from different laboratories for easier comparison. Other ursanes with potent anti-plasmodial activity are uvaol (***92***) and 2α-hydroxy-ursolic acid (***90***) isolated from *Baccharis dracunculifolia* with IC_{50} of 1·9 and 3 $\mu g\,mL^{-1}$ respectively against a chloroquine resistant K1 strain as well as 3-acetylpomolic acid (***101***) (IC_{50} 2·1 $\mu g\,mL^{-1}$) and pomolic acid (**100**) (IC_{50} 3·47 $\mu g\,mL^{-1}$) both isolated from *Markhamia tomentosa* (da Silva Filho *et al*. 2009; Tantangmo *et al*. 2010). Hence, ursane-type PT also provide a promising class of anti-plasmodials for future research.

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The lupane-type PT investigated for anti-plasmodial activity include betulinic acid (BA) (***129***) isolated from *Harungana madagascariensis* and *Zizyphus vulgaris* with IC_{50} values of 2·33 and 6·3 $\mu g\ mL^{-1}$ against W2 and 3D7 strains of *P. falciparum* respectively (Lenta *et al*. 2007; de Sá *et al*. 2009). A structural analogue of BA isolated from *Diospyros quaesita* highlights the importance of derivatization of the compound at C3 (true also for OA and UA) for potentiation of anti-plasmodial activity. The 3-caffeate derivative of BA (***122***) isolated from the plant was active against both chloroquine sensitive D6 and chloroquine resistant W2 strains of *P. falciparum* with IC_{50} of 0·86 and 0·61 $\mu g\ mL^{-1}$ respectively. This activity was enhanced with double acetylation of the caffeate (***121***) (IC_{50} 0·45 and 0·42 $\mu g\ mL^{-1}$, respectively) (Ma *et al*. 2008). Similar results were obtained with other derivatives such as messagenic acid A (***123***) and messagenic acid B (***124***) (trans and cis C27 coumaroyl derivatives of BA, respectively) isolated from *Gardenia saxatalis* which possessed IC_{50} of 1·5 and 3·8 $\mu g\ mL^{-1}$ respectively against a multidrug resistant strain of *P. falciparum* while the non-derivatized BA (also OA and UA) was inactive (Suksamrarn *et al*. 2003). Moreover, *cis* and *trans* C3 coumaroyl derivatives of BA (***138*** and ***139***) isolated from *Cornus florida* were also highly active derivatives against *P. falciparum* D10 with IC_{50} of 6·03 and 9·22 $\mu g\ mL^{-1}$ respectively (Graziose *et al*. 2012). Other naturally occurring lupane-type PT active against the K1 strain of *P. falciparum* are betulone (***126***) and lupenone (***127***) with IC_{50} of 1·32 and 2 $\mu g\ mL^{-1}$, respectively (Gachet *et al*. 2011). However, synthetic modifications of BA did not lead to profound increase in activity. Ziegler *et al*. (2004) reported the activities of methyl betulinate (***125***), betulinic aldehyde (***131***), betulinic acid amide (***132***), lupeol (***128***) and betulin (***134***) which were IC_{50} of 3·3, 6·2, 6·4, 11·8, and <12 $\mu g\ mL^{-1}$ respectively against *P. falciparum*. The more interesting finding of the study, however, was that BA, ***131*** and ***134*** resulted in a dose-dependent structural change in the membrane of non-parasitized erythrocytes. The compounds consequently prevented entry of *P. falciparum* merozoites into non-parasitized erythrocytes. These findings demonstrated that lupane-type PT may also restrict parasites' erythrocyte invasion *in vitro* via a mechanism that involves modulation of the erythrocytic membrane.

Few PT were investigated for possible *in vivo* anti-plasmodial activity based on their promising *in vitro* activities. The only oleanane-type PT that was investigated for *in vivo* anti-plasmodial activity was MA (***61***). Mice were infected with the lethal strain of *Plasmodium yoelii* and treated with a daily single intraperitoneal dose of 40 mg kg^{-1} body weight (bw) MA. As found in the *in vitro* studies, MA demonstrated a static effect on the parasite with accumulated schizonts in the erythrocytes of the infected mice. However, the treated mice consistently maintained lower levels of parasitaemia and remained immunoprotected against further infection with the parasite after 40 days (Moneriz *et al*. 2011*b*). Further analysis of the possible mechanism of action of MA suggested a multi routed mechanism involving the inhibition of a number of proteases necessary for the growth of the parasite. Other binding sites for MA, which include the *Plasmodium* phospholipase, were putatively proposed in an *in silico* analysis (Moneriz *et al*. 2011*c*). This remarkable *in vivo* activity demonstrated by MA calls for similar investigation on other oleanane-type PT especially those with even lower *in vitro* IC_{50} than MA such as epi-OA (***11***).

On the other hand, 3β-O-acetylursolic acid (***89***) was shown to suppress 94·01% of circulating *Plasmodium berghei* in mice (effective concentration not clear in the report). The compound was also less cytotoxic against HEK293 and HepG2 cell lines (Simelane *et al*. 2013). Furthermore, taraxasterol acetate (***115***) isolated from *Pluchea lanceolata* at 10 mg kg^{-1} bw suppressed 52·20% of circulating *P. berghei* in mice and showed 7 days extension of mean survival time (Mohanty *et al*. 2013). In another study, *in vivo* evaluation of betulinic acid revealed that the compound was ineffective in reducing *P. berghei* in mice even at 250 mg kg^{-1} bw day^{-1} (Steele *et al*. 1999).

One of the greatest limitation on the *in vivo* activity of PT, especially the less polar among them, is the hydrophobicity. Moreover, another limitation is the high cytotoxicity of some classes. For instance, Pristimerin (***1***) isolated from *Salacia leptoclada* was shown to have a selective index of <1 for P338 leukaemia cell lines (Ruphin *et al*. 2013), whereas 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (***5***) at 10 mg kg^{-1} bw was toxic to mice after just one day of administration (Figueiredo *et al*. 1998). The latter compound which was isolated from *S. kraussii* although being the most active of all PT against chloroquine-resistant *P. falciparum in vitro* (IC_{50} 0·037 $\mu g\ mL^{-1}$), was unable to clear *P. berghei* in mice treated with 1 and 5 mg kg^{-1} bw (Figueiredo *et al*. 1998). Therefore, bioavailability and cytotoxicity should be taken into account when further developing PT as possible anti-plasmodial agents is considered especially if the oral route is to be used. Moreover, these compounds could at least serve as structural backbones for synthesis of less toxic and more efficient compounds.

Anti-trypanosomal activities of PTs

Tingenin B (***7***), a quinone methide, is the most active reported PT against *Trypanosoma brucei brucei* and *Trypanosoma cruzi* with $IC_{50} < 0{\cdot}25\ \mu g\ mL^{-1}$ against each of the species. However, as observed

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with other compounds belonging to the same class, the compound was highly cytotoxic on MCR-5 cells (IC_{50} 0·45 $\mu g\ mL^{-1}$) (Maregesi *et al*. 2010). On the other hand, UA (***88***) has been reported in many studies to possess anti-trypanosomal activity with low IC_{50}. The compound isolated from *Strachynos spynosa* possessed an IC_{50} of 1 $\mu g\ mL^{-1}$ against *T. brucei brucei* (Hoet *et al*. 2007). Furthermore, in a study by Abe *et al*. (2002), UA from *Rosmanirus officinalis* with an MC_{100} of 40 $\mu g\ mL^{-1}$ was shown to be 86% more effective than the natural trypanocidal compound gossypol (Abe *et al*. 2002). Other structural analogues of UA were less effective or inactive against trypanosomes. The carboxylic group at C17 appears to be important for the anti-trypanosomal action of UA as evident in lower activities of uvaol (***92***) (aldehyde group replacing carboxyl) with an IC_{50} of 12·3 $\mu g\ mL^{-1}$ and α-amyrin (***95***) (methyl group replacing carboxyl) with IC_{50} of 48 $\mu g\ mL^{-1}$. Likewise, β-amyrin (***19***) (IC_{50} 54·2 $\mu g\ mL$) with CH_3 in place of COOH at C17 of OA (IC_{50} 2·9 $\mu g\ mL^{-1}$) lost anti-trypanosomal activity against *T. brucei brucei* (Hoet *et al*. 2007). The OH at C3 also appears to be equally important in the trypanosomal action of both UA and OA (Cunha *et al*. 2003; Taketa *et al*. 2004). This is confirmed by the loss in the activity against *T. cruzi* of UA in a mixture with OA with addition of acetyl group at C3 (***14*** and ***89***, respectively) of both compounds (Cunha *et al*. 2003). Moreover, oleanonic acid (***66***) and 3,11-dioxoolean-12-en-28-onic acid (***67***) (IC_{50} 113·62 and 173·9 $\mu g\ mL^{-1}$ against *T. cruzi*, respectively) which are similar in structure with OA but with adulterated C3 possessed no activity against the parasite (Cunha *et al*. 2003; Hoet *et al*. 2007; Leite *et al*. 2008). However, replacement of the C3 OH of OA with a polar group in saponin (***18***) did not lead to a loss in activity (Taketa *et al*. 2004). From these findings, it is evident that the presence (and/or property) of the C3 hydroxyl group and C17 COOH group are significant for the trypanocidal activity of the ursane and oleanane-type PT. The role of C3 OH may be, in part, to increase the polarity of the compound because glycosylation of the group in OA with a disaccharide (3-O-[β-D-glucopyranosyl-(1–2)-β-D-galactopyranosyl]) (***18***) or addition of potassium in UA (***93***) were found to maintain the activity against *T. brucei brucei* (IC_{50} 3·05 $\mu g\ mL^{-1}$) and *T. cruzi* (IC_{50} 4·26 $\mu g\ mL^{-1}$), respectively (Taketa *et al*. 2004; Cunha *et al*. 2006). However, substitution of the neighbouring carbon (C4) to C3 appears to counteract the effect despite the presence of additional polar groups as seen in the loss of activity of both brevicuspisaponin 1 and 2 (***102*** and ***103***) against *T. brucei brucei* where UA was most potent (Taketa *et al*. 2004). This suggests that in addition to increasing the polarity of the compounds, the nature and orientation of substituents at positions C3 and C17 may be involved directly in the activities of PT. Furthermore, the double bond between C12 and C13 may also play a role in the activity because friedelanol (***85***) lacking any double bond was inactive despite the presence of a C3 OH (da Silva Filho *et al*. 2004).

The anti-trypanosomal activity of the lupane group appeared in very few reports. Hoet *et al*. (2007) reported anti-*T. brucei brucei* activity of betulin (***134***), betulinic acid (***129***) and lupeol (***128***) with IC_{50} values of 4·0, 14·9 and 19·3 $\mu g\ mL^{-1}$, respectively.

In an *in vivo* setup, UA, OA and the potassium salt of UA (***93***) were potent against the lethal Y strain of *T. cruzi* in mice treated with daily intraperitoneal dose of 2 mg kg^{-1} bw. The treatment led to a reduction of parasite load in the infected rats more markedly by UA and the salt (75·7 and 70·4%, respectively) (Supplementary Table S1, available from http://journals.cambridge.org/PAR) (Cunha *et al*. 2006). In another study, da Silva Ferreira *et al*. (2010) reported 60 and 40% reduction in Bolivia strain of *T. cruzi* after treatment of infected rats with UA and OA at doses of 20 mg kg^{-1} bw day^{-1} orally. Findings of a later study demonstrated that the effectiveness of UA and OA treatment in *T. cruzi* infected mice is dependent on the bioavailability of the compound. It was observed that oral administration of the compounds (50 mg kg^{-1} bw day^{-1}) resulted in 79 and 76% decrease in parasitaemia respectively, while administration of the same concentration via the intraperitoneal route was not effective. Presumably, the intraperitoneal route achieved higher effective concentration of the compounds, which could have modulatory effects on pro and anti-inflammatory cytokines that resulted in an observed immunosuppression (da Silva Ferreira *et al*. 2013*a*). These effects may hence essentially counter the destructive effects of the compounds on the parasites since the immune system at some point of *T. cruzi* infection participate in parasite clearance (Tarleton, 2007). Hence, at low concentrations (which is achieved via the oral route due to low oral bioavailability of UA and OA or low intraperitoneal dose), the compounds are sufficient to destroy the parasites on their capacity or via other mechanisms.

On a final note, the anti-trypanosomal potential of PT is equally promising. Further research in this area should be directed towards screening more PT (especially the quinone methides) against various species of *Trypanosoma*. The need to investigate the compounds in animal models is also paramount because the compounds appear to facilitate parasite clearance via stimulation of host mechanisms which cannot be attained *in vitro*. In this regard, alternating the routes of administration is critical in order to provide a conclusive profile on the full potencies of PT as anti-trypanosomal agents.

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Anti-leishmanial activities of PTs

A number of studies have been conducted on the activity of PT against promastigotes and amastigotes of various *Leishmania* species. A range of saponin glycosides belonging to the oleanane PT isolated from *Maesa balansae* were very active against *Leishmania infantum* amastigotes with very low IC_{50}. The most active among them designated maesabalide III (***25***) possessed an IC_{50} of 0·007 $\mu g\ mL^{-1}$. Other maesabalides (***23***, ***24***, ***26***, ***27*** and ***28***) gave IC_{50} values of 0·014–0·046 $\mu g\ mL^{-1}$ (Germonprez *et al*. 2005). Oleonolic acid (***13***) isolated from *Salvia cilicica* also possessed activity with IC_{50} of 0·04 and 0·029 $\mu g\ mL^{-1}$ against promastigotes and amastigote of *Leishmania donovani*, respectively (Tan *et al*. 2002). Here also, the C3 OH of OA appears to play a crucial role in the activity as a conformational change tends to decrease the anti-leishmanial activity of the compound. This is because the activity of epi-OA (***11***) isolated from *Celaendendron maxicanum* was hundred-fold lower against the same parasite (IC_{50} 8·59 $\mu g\ mL^{-1}$) (Camacho *et al*. 2000). However, acetylation of the C3 OH group of OA may not cause a greater loss in activity. This is evident with acetylation of OA to form 3-OA acetate (***14***) which possessed an IC_{50} 2·49 $\mu g\ mL^{-1}$ against *Leishmania amazonensis* (Gnoatto *et al*. 2008). Other oleanane triterpenes with potent activities include hederacolchiside A_1 (***32***) (IC_{50} 0·061 $\mu g\ mL^{-1}$), β-hederin (***16***) (IC_{50} 0·26 $\mu g\ mL^{-1}$) and α-hederin (***15***) (IC_{50} 0·3 μg mL) from two hedera species against amastigotes of *Leishmania mexicana* (Ridoux *et al*. 2001; Tantangmo *et al*. 2010). On the other hand, glycyrrhitinic acid (GRA) (***33***), a derivative of β-amyrin (***19***) was also potent against *L. donovani* promastigotes *in vitro* with an IC_{50} of 4·6 $\mu g\ mL^{-1}$ (Ukil *et al*. 2005).

Among the ursanes, UA (***88***) isolated from *Salvia cilicica* appears to be the most active against both promastigotes and amastigotes forms of *L. donovani* and *Leishmania major* with low IC_{50} values of 0·0032–0·042 $\mu g\ mL^{-1}$ (Tan *et al*. 2002). However, other studies with UA reported much higher IC_{50} of 2·28 $\mu g\ mL^{-1}$ against *L. amazonensis* (Torres-Santos *et al*. 2004), 3·7 $\mu g\ mL^{-1}$ against *L. donovani* (da Silva Filho *et al*. 2009) and 4·55 $\mu g\ mL^{-1}$ against *Leishmania tarentolae* (Graziose *et al*. 2012). Some structural modification of UA led to reduction in activity as reported for 2α-hydroxy-ursolic acid (***90***) and uvaol (***92***) (IC_{50} 19 and 15 $\mu g\ mL^{-1}$ respectively against *L. donovani*) (da Silva Filho *et al*. 2009). On the other hand, a *bis*-(3-aminopropyl) piperazine moiety added to the carboxylic acid of 3β-acetylursolic acid (***89***) in compounds ***106–108*** retained the activity of UA against promastigotes of *Leishmania infantum* and *L. amazonensis* (IC_{50} 6–17 $\mu g\ mL^{-1}$) (Gnoatto *et al*. 2008). From the above findings, UA appears to be a potent anti-leishmanial agent against multiple species of the parasites. Because the investigated structural modification did not lead to an increase in activity, further modifications of the parent UA may be an experimental strategy for further development of ursane-type PT as anti-leishmanial agents. Other ursane-type PT with promising *in vitro* anti-leishmanial activity include pomolic acid (***100***) and 3-acetyl pomolic acid (***101***) from *Markhamia tomentosa* (IC_{50} 0·31 $\mu g\ mL^{-1}$ and 3·4 $\mu g\ mL^{-1}$, respectively) against *L. donovani* and synthetic N-{3-[4-(3-Aminopropyl) piperazinyl]propyl}−3-O-acetylursolamide (***105***) (IC_{50} 3·7 $\mu g\ mL^{-1}$ against *L. infantum*) (Gnoatto *et al*. 2008; Tantangmo *et al*. 2010).

In the lupane group, a few derivatives of BA were active against Leishmania although the parent compound was inactive in multiple studies. Betulinic acid acetate (***130***) and *trans* and *cis* 3-coumarol derivatives of BA (***138*** and ***139***) isolated from *Cornus florida* had IC_{50} values of 0·45, 5·14 and 1·36 $\mu g\ mL^{-1}$ respectively against *L. tarentolae* (Graziose *et al*. 2012). Moreover, dihydrobetulinic acid (DHBA) (***143***) from *Bacopa monniera* possessed an IC_{50} of 2·6 and 4·1 $\mu g\ mL^{-1}$ against *L. amazonensis* promastigotes and amastigotes, respectively (Chowdhury *et al*. 2003). Although a number of structural modification of the lupane-type PT led to loss in anti-leishmanial activity (Supplementary Table S3, available from http://journals.cambridge.org/PAR), future research on the group may be targeted towards different synthetic classes of the compounds and species of the parasite.

In an *in vivo* study, the anti-leishmanial activity of GRA (***33***) was further assessed where rats were treated with 50 $mg\ kg^{-1}$ bw day^{-1} (given three times, 5 days interval for 45 days) of the compound. The compound cleared the amastigotes form of the parasite from the liver and spleen of infected animals with a mechanism that involves decrease in the expression of mRNA for anti-inflammatory cytokines [interleukin (IL)-10 and IL-4] and an increase in the level of interferon-γ (IFN-γ) and tumor necrosis factors alpha (TNF-α) (Ukil *et al*. 2005). This comprehensively resulted in an increased immune response to the infection and clearance of the parasite via an nuclear factor kappa-B (NF-κB)-mediated mechanism. The mechanism through which GRA upregulate NF-κB was further described to involve multiple kinases and phosphatases (Ukil *et al*. 2011). Indeed, stimulation of the immune system has been deemed a rational strategy for the development of anti-leishmanial drugs (Santos *et al*. 2008). In a different study, oral and intraperitoneal administration of 10 $mg\ kg^{-1}$ bw DHBA to infected golden hamsters caused >90% reduction in parasite load in the spleen and liver of the infected animals. The compound was proposed to exert its effect via a mechanism that involves inhibition of DNA topoisomerases thereby essentially destroying the parasites

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(Chowdhury *et al*. 2003, 2011). As observed with other PT, the chemical entities on the C3 and C28 position of the lupeol-type PT is critical for the anti-parasitic activity of the group. Further analysis of the existing members of the group and structural manipulations to enhance activity is recommended.

Anti-nematodal activities of PTs

PTs were also investigated in a number of studies as possible therapeutic agents against lymphatic filariasis, onchocerciasis as well as conditions caused by other parasitic nematodes. Antifilarial activities of oleanane PT were reported against both human lymphatic filaria *Brugia malayi* and the rodent infective species. Oleanonic acid (***66***) and OA (***13***) isolated from the stem of *Lantana camara* were active against *B. malayi in vitro* with an LC_{100} of 31·25 and 62·50 $\mu g\ mL^{-1}$ respectively (Misra *et al*. 2007). Glycyrrhetinic acid (***33***) and its analogs (***34–37***) were also shown to be effective against the adult and microfilarial forms of *B. malayi*. The acyl derivatives (***38*** and ***39***) were inactive against both growth stages of the parasite, while the others showed IC_{50} values in the of 0·56–28·63 $\mu g\ mL^{-1}$ range against the microfilariae. However, against the adult worms, only the benzyl amide (***34***) and octyl amide (***35***) derivatives were active (IC_{50} 5·95 and 12·04 $\mu g\ mL^{-1}$ respectively) (Kalani *et al*. 2013).

In animal studies, the OA and oleanonic acid each administered at oral and intraperitoneal doses of 200 and 100 $mg\ kg^{-1}$ bw respectively had no effect on the circulating microfilariae of *B. malayi* in infected mastomys. However, against the adult worms, both compounds showed approximately 56% female worm sterility, although only OA had a filaricidal activity of 18·18% (Misra *et al*. 2007). Moreover, *B. malayi* infected jirds were treated with 100 $mg\ kg^{-1}$ bw doses of the *in vitro* active amide derivatives of GRA (***34*** and ***35***). The result showed that only the benzyl amide derivative possessed macrofilaricidal activity (54%) while the other was inactive (Kalani *et al*. 2013).

The oleanane-type PT, 3-*O*-acetyl aleuritolic acid (***75***) isolated from *Discoglypremna caloneura* was active against *Onchorcerca gutturosa* worms. The compound was found to reduce the motility and viability of the worms up to 57·1 and 64·8%, respectively. The reduction in viability was found to be 33·3% more than that of amocarzine and hence compound ***75*** was considered an interesting compound against filarial infections (Nyasse *et al*. 2006).

Although the volume of research on the antifilarial activities of PT is not large, available data suggest them to be potent against different filariid. Hence, future screening of other PT against filariasis may be worthwhile.

Betulin (***134***) from *Schefflera vinosa* as well as OA and UA from *Miconia langsdorfii* were tested for schistomicidal activities. Among the three compounds, only ***134*** led to the mortality of the adult worms of *Schistosoma mansonii* at concentrations of 100 μM (25% mortality) and 200 μM (50%) after 120 h of incubation (Cunha *et al*. 2012). Further research on this subject area should focus on testing newly isolated and available PT on different species of *Schistosoma* to compliment the library of biological activities of the group as future anti-parasitic agents.

OA isolated from *Calendula officinalis* was investigated for possible nematocidal activity against the mice intestinal parasite, *Heligmosomoides polygyrus*. The compound alongside other derivatives exhibited >50% growth inhibition of the larvae incubated with 70 $\mu g\ mL^{-1}$ of the compounds *in vitro*. The mechanism through which OA and related PT reduces the viability of *H. polygyrus* was later shown to involve modulation of the pattern of larval antigen glycosylation which appears to lead to a robust increase in cytokine production in mice infected with larvae incubated with the compound (Doligalska *et al*. 2013). Because anti-filarials act via an immune-mediated mechanisms (Hoerauf *et al*. 2011), and PTs were shown to modulate the immune system, PTs are logical candidates for *in vivo* screening as anti-filarial drugs.

MA was also investigated for possible action against the *Trichinella*, the causative agent of trichinellosis in humans. Against the mammalian infective *Trichinella zimbabwensis*, the compound orally administered once on 25 dpi or twice on 25 and 32 dpi cleared >90% of the parasite's larvae. This was achieved at a lower dose (2·5 $mg\ kg^{-1}$ bw) compared with the anthelmic drug fenbendazole (7·5 $mg\ kg^{-1}$ bw) which gave similar efficacy (Mukaratirwa *et al*. 2016). Hence, MA has shown promising activity against *Trichinella* and therefore screening of other PT against this parasite will be worthwhile.

Against the plant nematode *Meloidogyne incognita*, camarinic acid (***110***) activity was similar to that of a standard nematicidal drug, furadan, at the same concentration of 1 $mg\ mL^{-1}$. The compound which was isolated from *Lantana camara* led to 100% larval mortality after 24 h exposure (Supplementary Table S1, available from http://journals.cambridge.org/PAR) (Begum *et al*. 2000). Later studies on this plant showed it to be a repository of PT with varying degrees of nematicidal activities. Lantanilic acid (***46***), camaric acid (***45***) and OA (***13***) from the plant caused 98, 95 and 70% *M. incognita* larval mortality respectively at 5 $mg\ mL^{-1}$ concentration (Qamar *et al*. 2005). Furthermore, camarinin (***43***), lantanolic acid (***44***), UA, pomolic acid (***100***), lantacin (***114***), camarin (***77***) and lantoic acid (***111***) from the same plant all caused 100% larval mortality at 1 $mg\ mL^{-1}$ concentration after 48 h of exposure. Compounds ***43***, ***44*** and UA (***88***) proved to be comparatively more potent with 90, 10 and 10% larval mortality at 2 $\mu g\ mL^{-1}$ after 72 h exposure (Begum

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et al. 2008). In a different study with *Cordia latifolia*, cordinoic acid (***112***) isolated from the plant at 5 mg mL^{-1} concentration led to 100% *M. incognita* larval mortality after 24 h exposure (Begum *et al*. 2011). On the other hand, polygalacic acid (***48***) and bayogenin (***49***) and their saponins ***50–60*** isolated from *Microsechium helleri* and *Sicyos bulbosus* were active against *Meloidogyne javanica* that also affects plants. Among the compounds, those with a xylose residue attached to the second rhamnose residue at the substituent on C28 (***50–53***) were found to be inactive while the others inhibited >74% of the parasite's larvae growth at various concentrations. Moreover, bayogenin which differs from polygalacic acid only in the absence of an OH group at C16 of the latter molecule was the most active together with saponin ***58***. Both compounds immobilized 100% of the parasite's larvae at 0·5 $\mu g\ mL^{-1}$ concentration (Hernández-Carlos *et al*. 2011). From the above findings, it is clear that the activities of PT and their saponin against plant nematodes are promising and warrant further investigation.

Activities of PTs against other parasites

Toxoplasma: Maslinic acid (***61***) isolated from *Olea europaea* inhibited the infectivity of Vero cells by *T. gondii* tachyzoites with an ID_{50} of 3·78 $\mu g\ mL^{-1}$ after incubation for 48 h. Moreover, the compound at a concentration of 50 μM inhibited the motility of 100% of the parasites. The compound was also shown to inhibit key parasite proteases thereby effectively blocking parasite entry into the cells (De Pablos *et al*. 2010). This dual effect (inhibition of motility and entrance into cells) of MA on *T. gondii* is interesting as therapeutic approach and hence calls for further screening alongside other PT.

Trichomonas: Only one PT, hederagenin (***47***), isolated from *Cussonia holstii* was investigated for activity against *Trichomonas viginalis*. The result indicated high *in vitro* activity with an IC_{50} of 1·32 $\mu g\ mL^{-1}$ (He *et al*. 2003). Hence, PT could be suitable candidates for future screening as anti-trichomonas agents.

Toxicity aspects

One of the disadvantages of using PT as therapeutic agents has been known to be associated with high cytotoxicity (Dzubak *et al*. 2006). However, at low concentrations, some of these PT have proved to be therapeutic (Liu, 2005). Moreover, cytotoxicity studies of some PT, for example MA, reported *in vivo* safety both in acute and chronic treatments (Sánchez-González *et al*. 2013). In another study, BA was found to have selective toxicity against cancerous cells but not normal cells (Zuco *et al*. 2002). Hence, since PT are selective to different cells lines, further toxicity assessments and *in vivo* safety studies of the most active compounds is warranted.

CONCLUSION AND FUTURE DIRECTIONS

Various research findings from plants of different parts of the world have revealed that PT represent a promising group of phytochemicals with good therapeutic potential against a number of parasitic diseases. However, the studies on the anti-parasitic potential of PT are at preliminary proof of concept stages with only 22 out of the total 191 PT having been investigated in animal models. This underscores the need to re-focus research efforts on *in vivo* studies of PT against different parasitic infections which may pave the way for further clinical trials and drug development.

On a general note, it is noteworthy that the PT seems to be more promising for future development as anti-malarial agents. This is evident by the propensity of anti-plasmodial studies of PT as well as the potent activities reported for most of the tested PT. However, this does not exclude the possibility of developing therapeutically active PT against other parasites, especially the less studied parasites such as toxoplasma, trichomonas, schistosoma and nematodes.

Another pertinent finding from this review is that quinine methides are the most biologically potent PT with respect to parasitic diseases especially those caused by malaria parasites. Unfortunately however, this class of the compounds also seems to be the most toxic among all the PTs. Thus, studies on quinine methides to target synthetic modifications at various positions of the parent backbone with the aim of minimizing their cytotoxicity, whilst maintaining the anti-parasitic activities should be conducted. In fact, this should be the next step to be taken if research efforts on quinine methides are to be geared along the drug development process.

SUPPLEMENTARY MATERIAL

The supplementary material for this paper can be found at http://dx.doi.org/10.1017/S0031182016000718

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