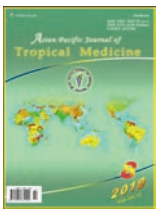

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Pharmacological and analytical aspects of artemisinin for malaria: Advances and challenges

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ABSTRACT

Malaria remains a major tropical health burden owing to the development of resistance and decreased sensitivity to the frequently used conventional antimalarial drugs. The drug like artemisinin possesses potent antimalarial activities, but has some limitations. Therefore, new strategies are to be implemented for optimal utilization of artemisinin to improve its therapeutic effectiveness and to overcome its limitations. The present review focuses on present scenario of malaria and pharmacological as well as analytical aspects of artemisinin. Data from 2000 to 2018 were collected from NCBI for understanding the various analytical techniques used for estimation of artemisinin. This review will reveal the facts about artemisinin which can be utilized to develop novel drug delivery system either in a combination or as alone for the wellbeing of the patients suffering from malaria.

1. Introduction

Malaria is one of the most serious tropical diseases, it requires therapeutic attention. Malaria stood a major global health complication with the high probability of development of resistance towards majority of conventional and frequently used anti-malarial agents. This problem is enhanced due to decreased ineffectiveness of drugs like chloroquine against *Plasmodium (P.) vivax*. The malaria is a protozoal disease mainly caused by four species of *Plasmodium (P. falciparum, P. ovale, P. malaria, P. vivax)* and other species like *P. knowlesi, P. bhergie, etc.* However, *P. knowlesi* and *P. bhergieare* are parasites which cause malaria in animals other than human beings. Whereas *P. berghei* is used as *in-vivo* antimalarial experimental model for the evaluation of anti-malarial activity of unknown test substances[1,2]. An *Anopheles* mosquito is the carrier for malarial parasite. When parasite enters in the human blood stream, it produces the symptoms like fever, chills, headache fatigue, nausea, vomiting, diarrhoea, occasionally coma and death may occur, *etc*[3]. The treatment of malaria is very essential but still there are many limitations and challenges in the management and control of malaria. Artemisinin is one of the widely used anti-malarial drugs with good therapeutic potential against malaria. It is sesquiterpene lactone having endoperoxide group obtained from Chinese herb *i.e. Artemisia (A.) annua*. The present review highlighted the role of artemisinin in the management of malaria along with its pharmacological and analytical aspects. The literature selection flowchart is shown in Figure 1.

2. Epidemiology of malaria

According to the 11th World Malaria Report 2018 by World Health Organisation, the global malaria cases found in 2016 and 2017 are 217 and 219 million respectively[4,5]. In the year 2017, there were

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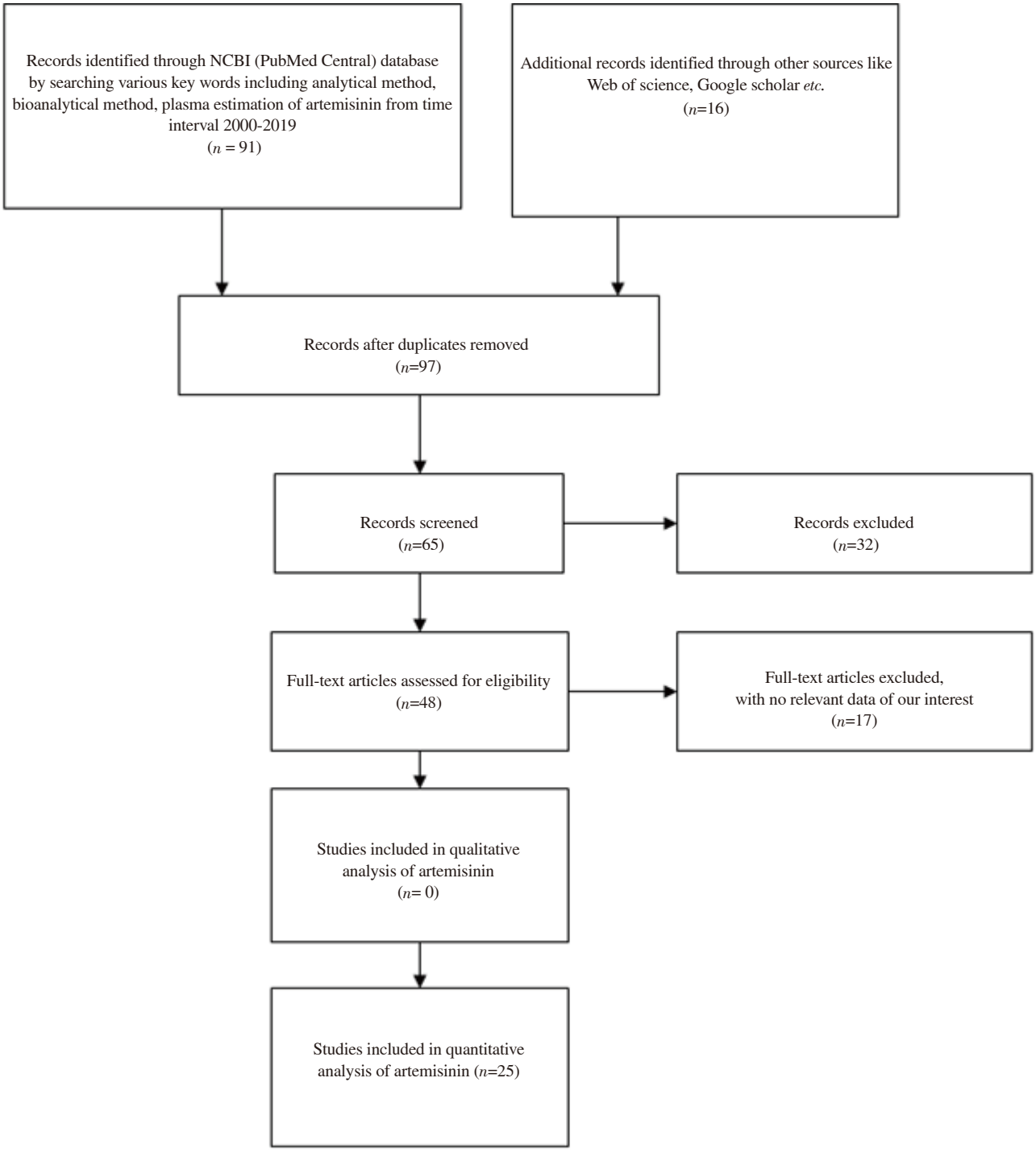

Figure 1. The flowchart of the literature selection process.

435 000 deaths estimated from malaria globally. Children below 5 years were most affected by malaria to become the particularly vulnerable group across the world. About 266 000 deaths were observed, *i.e.* 61% of all malaria deaths worldwide from this age group[5,6]. Further, about 80% of deaths were recorded from 17 countries including African Region and India, of which, 7 countries accounted for 53% deaths around the world (Burkina Faso 6%, Democratic Republic of the Congo 11% , India 4%, Niger 4%, Nigeria 19 %, Sierra Leone 4%, United Republic of Tanzania 5%)[5,7,8]. These data indicated that malaria still requires a great attention.

3. Current challenges in the management of malaria

The present world is experiencing vulnerable effects of malaria and its complications. The epidemiology of malaria is increasing day by day. The treatment of malaria becomes a big task as it has many challenges. The examples of such challenges are, urban malaria, parasite's drug resistance, malaria in pregnancy, resistance towards insecticides, *etc*[9]. The treatment was using current antimalarial drugs in combination with artemisinin and its derivatives as a 'partner' drug. This strategy is known as artemisinin combination therapies and this is considered as the choice of treatment in

malaria. But as per the recent reports, the resistance has been found to be developed to artemisinin as well as their derivatives. This raised the considerable concern in the utilization of artemisinin for the management of malaria. Therefore, the public health care policies re-focussed on maximising therapeutic effectiveness and reducing chances of development of resistance even on its long-term utilization for the treatment of malaria[10]. Hence, either new anti-malarial drugs are to be evaluated or bio-enhancers should be used along with artemisinin so as to counteract its limitations.

4. Artemisininin

The Nobel Prize was won by Youyou Tu and other Chinese scientists (2015) in the field of physiology or medicine research for discovering artemisinin and its derivatives like artemether and artesunate. These anti-malarial drugs saved millions of people across the globe[11]. Artemisinin is derived from *A. annua* extracts. It is mainly used against highly drug resistant strains causing malaria. The recent reports have shown that artemisinin also has potent anticancer activity. Nevertheless, artemisinin is safe and less expensive, too[12] .

It is a huge scope in the development of various novel drug delivery systems and evaluation of further therapeutic potential of artemisinin and its derivatives. Certain facts associated with artemisinin are given in Table 1.

Artemsinin is natural molecule obtained from *A. annua* discovered in year 1967 by research conducted by the Chinese Academy of Medical Sciences in Beijing by Youyou Tu under the title Project 523[22]. It is one of the most potent drugs for the treatment of malaria which acts on both blood stage and liver stage schizonts, but rapidly affects blood schizonts. It is active against almost all species of *Plasmodium* including resistant *P. falciparum*. After administration of initial dose of 20 mg/kg, parasitemia can be cleared within 48 h. The pharmacodynamics aspects of the drug are not clear but the probable action of artemisinin towards the parasite may be due to the cleavage of endoperoxide bridge. This leads to release of highly reactive species of free radicals which damages parasite proteins and offers rapid killing. But complete clearance of this drug is slow because of its low half-life less than 1 hour due to certain points like low half-life, Biopharmaceutics Classification System (BCS) class [[solubility, slow clearance, recrudesce of parasitemia, low bio-availability, *etc*. Artemisinin is not recommended alone as monotherapy and its prophylaxis usage is irrational. All these mentioned advantages and limitations need to be considered while initiating the research associated with artemisinin so as to fulfil the related lacunas.

4.1. Mechanism of artemisinin action

The exact mechanism of artemisinin action is still debatable. The probable mechanism of action includes killing of parasite by releasing highly reactive free radicals responsible for lysis of parasite[19]. These carbon-centred free radicals were generated through heme-mediated decomposition of the endoperoxide bridge, which binds to the membrane proteins and lipid peroxidation takes place. This damages the endoplasmic reticulum and inhibits protein synthesis in parasite. It results in lysis of parasite and shows its anti-malarial activity.

Table 1. Biopharmaceutic characteristics of artemisinin and its derivatives.

Parameter	Artimisinin	References
Antimalarial activity	Rapid blood schizonticide and acts on wide range of stages; Mainly sensitive and resistant *Plasmodium falciparum;* Acts wide range of stages from ring form to schizonts but does not kill hypnozoites; Acts on gamates	[11]
Solubility	Artimisinin: Poorly soluble in water and oil. Artersunate: water soluble. Artemether: oil soluble or lipid soluble	[13]
BCS class	Class [[drug	[14]
Pharmacokinetic properties	Rapid onset of action and fast clearance. T ½ less than 1 hour. Lipid soluble; Artisunate: t ½ 30 min-60 min. Arteether: t ½ 23 h. Artemether: t ½ 3 h-10 h.	[15,22]
Clearance of parasitemia	< 48 h.	[16]
pKa	Strongest basic-4.4.	[17]
Dose	Initial dose-20 mg/kg. later 10 mg/kg up to 6 d; Artisunate: 50 mg-60 mg. Artemeether: 40 mg-80 mg (inj). Arteether: 150 mg/2 mL ampoule; WHO (2006) recommended therapy: 1st day-3.2 mg/kg. Followed by 1.6 mg/kg for next 4 d	[3,18]
Mechanism of action	Not clear; Iron mediated cleavage of endoperoxide bridge; Release highly reactive free radicals lead to lysis of parasite	[19]
Side effects	Few and mild headaches, nausea, vomiting, abnormal bleeding, dark urine, Itching; Drug fever in some	[3]
Resistance	So far no resistane among *Plasmodium falciparum* patients to artimisinin has been noted (Tripathi); However, it is just the beginning; Now it can be observed in Thai-Myanmar regions where it started spreading	[18,20]
Monotherapy/ combination	Monotherapy is not recommended due to its shorter t½.. Prophylaxis is irrational; Combination is preferable	[21]
Comments	Rapid action and most efficacious. Recommended in combinations (Biamonte); Safe & cheap; Used in acute cases; High rate of parasite recrudescence	[3,12,13,15,22]

4.2. Analytical & bio-analytical methods used for artemisinin quantificationin research publications of NCBI from 2000 to 2018

Many analytical and bio-analytical methods used for artemisinin quantification (Method: Research publications in NCBI from 2000 to 2018) are summarized in Table 2. The abbreviations are listed here: methanol extracts of artemisinin (AM), arteannuin-B (AB), artemisinic acid (AA), hexane extraction of *A. annua* (AH), acetonitrile (ACN), artemisinin (ART), trifluoro acetic acid (TFA), artesunate (AS) , dihydroartemisinin (DHA), water H_2O, petroleum ether–acetone extract of *A. annua* (APE-A), *n*-hexane extract of *Artemsias* spp. (AnH), artemether (AM), dihydroartemisininGlucoronide (DHA-G), Formic acid (FA), chloroform ($CHCl_3$), hexane (H), ethyl acetate (EA), methyl alcohol (MeOH), artemether (ARM), formic acid (FA), triple stage quadrupole (TSQ), mass spectrometer (MS), β-arteether (AE), limit of detection (LOD), limit of quantification (LOQ).

Conventional and advanced chromatographic techniques like thin layer chromatography (TLC), Gas chromatography (GC), High Performance Liquid Chromatography (HPLC-UV), GC with flame ionization detection (GC-FID) have been used for quantification of artemisinin. HPLC with evaporative light scattering detector (HPLC-ELSD) was used in specific condition. Other than chromatographic techniques, icELISA-Indirect Competitive Enzyme linked immune sorbet assay and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) were used for estimation of artemisinin in different matrices. Consequently, hyphened techniques HPLC-ESI-MS/MS-HPLC with electrospray (ESI) ion mass spectrometry, UPLC techniques and their corresponding methods have been applied in the analysis of artemisinin.

Chromatographic techniques are frequently used for determination of artemisinin and its metabolites dihydroartemisinin in the different matrices. Moreover, isocratic as well as gradient mode was used for quantitative analysis of artemisinin. Limit of detection and limit of quantification were significantly established. As per bio-analytical aspect, hyphened techniques like high-performance liquid chromatography/tandem mass spectrometry LCMS/MS were used for determination of artemisinin in biological fluid. The frequent utilization of chemicals, drug candidate and solvents in chromatographic methods in pharmaceutical products has become a challenge to green chemistry. In the determination of artemisinin in different matrices by chromatographic methods requiring toxic solvents like methanol, acetonitrile and buffer are used which are also the environmental polluents. Therefore, researchers should think about utilization green solvents in the estimation of artemisinin in pharmaceutical formulations.

5. Artemisinin resistance as a challenge

The artemisinin is under risk due to occurence of the resistance in *P. falciparum* parasites in the Southeast Asian countries[48]. The malaria is a deadly tropical disease affecting many countries. There are many challenges in the treatment of malaria, of which the resistance is one of the most vital challenges. The conventional drugs such as chloroquine, mefloquine, pyrimethamine, quinine, sulfadoxine, *etc*. became inactive or less active against the parasite *P. falciparum* due to resistance. Moreover, the malarial parasite developed resistance against the most potent antimalarial drug-artemisinin as well[49]. This is a big challenge in front or the healthcare professionals in the management of malaria. However, artemisinin combination therapy is considered as the first-line therapy in the regions where artemisinin alone has developed the resistance, but this strategy also failed and did not worked more efficiently. Therefore, the death rate got increased in these areas. Thus the new combinations with artemisinin are under clinical trials so as to counteract this problem and minimise the morbidity as well as mortality in affected areas. The study reported that G625R as a probable mutation was responsible for artemisinin resistance along with *R539T* gene mutation. Previously reported mutation was *kelch13* alteration. The exact evaluation of this newly reported mutation is still unclear. Though lumefantrine and artemether are recommended combination in northeast India, pyrimethamine plus sulfadoxine with artesunate combination is still used in other regions of India[50]. Moreover, in order to tackle the alarming artemisinin resistance in malaria, WHO issued its *Global plan for artemisinin resistance containment (GPARC) and emergency response to artemisinin resistance in the Greater Mekong Subregion*. The major objective of these plans are to control and then complete eradicate artemisinin resistance malaria across the globe[51]. Recently, Nobel Laureate Youyou Tu announced that her team has proposed solutions to the problem of artemisinin resistance, giving new proofs that artemisinin is still "the best weapon" against malaria[52]. Furthermore, the alarming rate of artemisinin resistance need extensive attention of researchers so as to get the solution for effective and prompt management of malaria across the world.

6. Future perspectives of artemisininin

As there are many complex limitations in the utilization of anti-malarial drug in the treatment of malaria, it is very essential to discover either new anti-malarial agents or to modify the current therapeutic approaches. Moreover, the combination therapy especially with artemisinin using appropriate partner possessing anti-malarial activity or bio-enhancing potential can be the best approach. The new drug discovery from natural/semisynthetic/synthetic origin with or without chemical modifications can make the things in favour of malarial patients therapeutically. Such chemical modifications can also be applicable to already existing anti-malarial

Table 2. Analytical & bio-analytical methods used for artemisinin.

S/No	Matrix	Technique	Mobile phase	Column	Flow rate	Column temp	λ(wave length)	LOD	LOQ	References
1	AM, AB, AA	RP-TLC	0.2% TFA in water/ACN (35:65, v/v)	RP-18 F254 S thin-layer chromatographic plates		Room temperature	426 nm in absorption reflectance mode	AM: 40 ng, AB: 80 ng, AA: 20 ng	AM: 100 ng, AB: 200 ng, AA: 50 ng	[23]
2	AH and reconstituted with ACN	HPLC–ELSD	TFA: ACN (65:35) pH 3.0-3.5	HPLC: C18-RP 250 mm×4.0 mm ID (5.0 µm pore size) ELSD: PL-ELS1000 ELSD.	1.0 mL/min ELSD: nitrogen flow at 0.8 L/min	ELSD: evaporative temperature of 80 ℃, nebulizer at 75 ℃.		ART: 50 ng, AB:100 ng, AA: 500 ng.		[24]
		GC–FID		Rtx-5 crossbond 100% dimethyl polysiloxane (Resteck Corp), (15 m×0.25 mm ID, 0.25 µm film thickness)	Column flow: 1.50 mL/min	195 ℃, injector at 240 ℃, and FID temperature set at 300 ℃		ART: 30 ng, AB: 5 ng, AA 4 ng.		
3	AS and DHA in human plasma	RP-HPLC	ACN-0.05M acetic acid adjusted to pH 5.2 with 1.0 M NaOH (42:58, v/v)	Hypersil C4, 250 mm×4.6 mm I.D., 5 µm particle size.	1.50 mL/min			For both AS and DHA: 4 ng/0.5 mL	For both AS and DHA: 10 ng/0.5 mL	[25]
4	APE-A	HPLC-ELSD	HPLC: H_2O: ACN (40:60 v/v)	HPLC: Agilent C18 column	HPLC: 1mL/min. ELSD: nebulizer-gas flow rate of 2.0 L/min	ELSD: drift tube temperature of 70 ℃		< 40 µg/mL	< 100 µg/mL	[26]
5	AnH	RP-HPLC	FA 0.2% (v/v): ACN (50:50 v/v)	ACE-5 C18 column (5 µm; 250 mm ×4.6 mm).	1 mL/min	30 ℃		ART: 0.03 µg/mL	ART: 0.1 µg/mL	[27]
6	Pure forms of AS, AM, DHA, DHA-G, prepared in methanol (1 mg/mL) estimated in sheep plasma (*in–vitro*)	LC-MS/MS	HPLC: Mobile phase A: 5 mM ammonium formate plus 0.15% (v/v) FA in ultra-pure water. Mobile phase B: 0.15% (v/v) FA in ACN	HPLC: 2.1 mm×20 mm Atlantis T3 3-m HPLC coupled with API 365 triplequadrupole mass spectrometer equipped with a turbo ion spray interface, operated in positive ionisation mode	0.3 mL/min	25 ℃			LLOQ: DHA-G, DHA and AS: 10.2 ng/mL, AM: 93.8 ng/mL	[28]
7	AS and DHA in human plasma	LC–MS/MS	ACN-ammonium acetate 10 mM pH 3.5 (50:50, v/v)	Post column infusion						[29]
8	$CHCl_3$, H, ACN and EA extracts of *Artemisia annua* leaf	HPLC-ELSD with RI detector HPLC-RI	HPLC-UV: a) ACN/aqueous ammonium hydroxide (40:60, v/v), pH adjusted with acetic acid; b) 50:30:20 (%, v/v) ACN:H_2O:MeOH	1) XR-ODS 50 mm×2 mm with a 2.2 µm deactivated type B silica, 12 nm pores; 2) Betasil C18 5 m 250 mm×4.6 mm	1) 0.5 mL/min 2) 1.0 mL/min		Gradient (a) UV-213 nm Isocratic (b)UV- 210 nm	0.009 mg/mL 0.001 mg/mL	0.020 mg/mL 0.009 mg/mL	[30]
			ELSD: a)ACN/aqueous ammonium hydroxide (40:60, v/v), pH adjusted with acetic acid; b)50:30:20 (%, v/v)		Nitrogen flow: 3.6 L/min	55 ℃	Gradient ELSD (a) Isocratic ELSD (b)	0.001 mg/mL 0.020 mg/mL	0.100 mg/mL 0.100 mg/mL	
	$CHCl_3$, extract of *Artemisia annua* plant		ACN:H_2O:MeOH HPLC-RI: 60:40 (%, v/v) ACN:H_2O	PhenomenexGemini 5µm C18 11 nm 250 mm×4.6 mm	1.0 mL/min	Ambient temperature		0.025 mg/mL	0.1 mg/mL	
9	ARM, AS, DHA (pure) in plasma	LC-MS/MS	LC: 20 mM ammonium formate in ultrapure water (buffer A) and ACN (Solvent B), both containing 0.5% FA. LC-MS/MS: gradient elution of mobile phase	2.1 mm×50 mm Atlantis dC18 3 µm	0.3 mL/min			DHA: 0.5 ng/mL AS: 0.5 ng/mL AM: 3 ng/mL	DHA: 0.75 ng/mL AS: 1.5 ng/mL AM: 5 ng/mL	[31]
		MS		TSQ Quantum Ion MS						
10	Pure ART in human plasma	Spectrophoto-meter	DMSO solubilized ART (17.7 nmol/mL) in 0.05 N NaOH				291 nm			[32]
11	ART concentration in flowers, leaves, roots and stems of *Artemisia* spp (toluene extract)	HPLC	45% (v/v) MeOH (Sigma) and 55% 0.01 M sodium phosphate buffer (pH 7.0)	Zorba×SB C18 column (150× 4.6 mm ×5 µm)	1 mL/min		260 nm			[33]
12	Toluene extract of leaves of *Artemisia annua*	TLC	*n*-hexane and diethyl ether (6:5, v/v)	precoated silica gel TLC plates 60F254 (Merck,Darmstadt, D), 10 cm×20 cm					0.45%	[34]

Table 2. Analytical & bio-analytical methods used for artemisinin (continued).

13	ARM both as a bulk drug and in capsule formulations	HPLC	ACN and buffer in the ratio 65:35 of pH 6.5 adjusted with tryethylamine	HypersilOctadecylsilane (ODS) column, (250×4) mm, 5 μm particle size of packing, pore size 120 A			210 nm	21.83 mg/mL	750 mg/mL	[35]
14	Ethane extract of *Artemisia annua*	HPLC	Mixture of MeOH and phosphate buffer (5.0 mM; pH: 6.3) (45/55, v/v)	reversed phase RP-18 LiChroCART column (250 mm×4 mm I.D; particle size: 5 m)	1 mL/min	35 ℃	260 nm	2.73 μg/mL	10.0 μg/mL	[36]
15	Ethyl acetate, hexane, ethanol and a hexane-ethyl acetate (95:5, v/v) mixture	HPLC	Isocratic mobile phase of ACN:H_2O (50:50, v/v) followed by an ACN:H_2O (80:20, v/v)	250 mm×4.6 mm×5 μm SunFire C18 column	1 mL/min	25 ℃	192, 200, 205, 210 and 215 nm, with 210 nm			[37]
16	ART in atemisinin leaves based tea, biscuits, porridge	HPLC-MS/MS	HPLC: 0.01 mol/L ammonium acetate in water (solvent A) and ACN (solvent B)	125 mm×2 mm, 5 μm particles	0.2 mL/min					[38]
		MS		1200 series LC system, coupled to a 6410 triple quadrupole mass spectrometer	8 mL/min and a pressure of 276 kPa	Nebulizer gas with a temperature of 340 ℃				
17	ART and DHA in human plasma	HPLC-MS/MS	HPLC : Gradient elution with Mobile phase A: 0.1% ammonia in water; and Mobile phase B: ACN. MS detection: positive ionization mode.	Xbridge C18 column (50 mm×2.1 mm i.d., 3.5 μm particles).	To 0.35 mL/min	40 ℃ Atmospheric pressure chemical ionizationoperating at 450 ℃				[39]
18	ART, DHA, ARM and AS compared pure and marketed preparations	HPLC and icELISA	60% aqueous acetonitrile	C18 reverse-phase column (250 mm×4.6 mm, 5 μm)	1 mL/min	210 nm				[40]
19	AE	HPLC	Isocratic elution of methanol-ammonium acetate (pH 4; 10 mM) (80:20, v/v)	C18 column (125 mm ×4 mm, 5 μm)	0.45 mL/min	32.5 ℃		AE: 40 μg/mL	AE: 120 μg/mL	[41]
20	ARM pure	HPLC ELISA	ACN and 0.5% acetic acid (70/30, v/v)	C18 reverse-phase column (250×4.6 nm, 5 μm particle size)	1 mL/ min		210 nm			[42]
21	ARM and its metabolite DHA in plasma	LC-MS/MS	Gradient elusion mode of 10mM ammonium formate at pH=4.0 and acetonitrile (0.1% formic acid)	Acquity HSS C18 column (100 mm×2.1 mm, 1.8 μm)	0.3 mL/min				LOQ: 0.5 ng/mL for both ARM & DHA	[43]
22	AS pure	DRIFTS HPLC-ESI-MS/MS	H_2O/ACN/MeOH (30:35:35 v/v/v) containing 0.1 % formic acid.	XBridge C18 column (50 mm×2.1 mm, 5 μm) coupled with an XBridge C18 guard column (10 mm×2.1 mm, 5 μm)	350 μL/min	25 ℃		0.13 mg/100 mg	0.39 mg/100 mg	[44]
		ESI	Ion spray voltage of 5 500 V			Source temperature of 400 ℃				
23	ARM pure	HPLC	ACN and 50 mM potassium dihydrogen orthophosphate buffer (pH 3) in a ratio 55:45	100 mm×2.1 mm, 3.5 μm C18 column	0.8 mL/min	40 ℃	210 nm	0.000 3 mg/mL	0.005 mg/mL	[45]
24	ART	UPLC	Isocratic: 60% of a 0.1% aqueous solution of formic acid and 40% ACN	C4 100 mm×2.1 mm column	0.4 mL/min			0.005 μg/mL	0.010 μg/mL	[46]
25	ART pure	Direct analysis in real time -mass spectro-metry (DART-MS).				300 ℃				[47]
	Aretemisia annua plant extracts	HPLC	ACN/ H_2O/acetic acid = 50/50/0.1 (v/v/v)	Reverse-phase C18 column (Thermo Fisher Scientific Inc., 4.6 mm×250 mm, 5.0 μm, USA)	1.0 mL/min		210 nm	0.5 μg/mL	2 μg/mL	

agents. Consequently, the development of hybrid compounds, re-evaluation of commercially available therapeutic agents that have been approved for clinical utilization for any other morbidity and advanced molecular modelling by docking studies and virtual screening technology may play an important role. Nevertheless, finding a potent and appropriate bio-enhancer for artemisinin can also make remarkable significance in the therapeutic management of malaria[1]. Using these strategies and suitable approaches with specificity against malarial parasites optimum control can be achieved on tropical diseases like malaria.

Conflict of interest statement

We declare that we have no conflict of interest.

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