

# Novel Study On Antimalarials And To Develop New Drugs For Resistance Of Plasmodium Species

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## Abstract

Malaria is a major health problem and about half of the World population still at risk in 95 malaria endemic countries. Due to limited health facilities and high cost and unavailability of artemisinin-based combination therapies (ACTs), a fifth of malarial patients in these countries use traditional medicinal plant remedies. In current review, we have highlighted the antimalarials plants used in various societies across Pakistan. In Pakistan, a total of (77) species of (63) genera belonging to (35) families have been traditionally used for the treatment of malaria. Among these, several plants have been investigated phytochemically and pharmacologically across the globe for antiplasmodial activity and have shown a good antiplasmodial activity. In the other plants on chemotaxonomic basis, several plants need evaluation for antimalarial activity. In most of the ethnobotanical studies conducted across Pakistan, essential information about formulation, dosage, time and duration of use of remedies has not been documented. However, they can be investigated in order to claim their antimalarial potential. Although, these medicinal plants have been used over history for treatment of malaria but, evaluation of their pharmacological activities is essential in order to establish their mode of action, safety, quality, efficacy and to find novel antimalarials and to develop new drugs for resistance of Plasmodium species.

**Keywords:** Traditional remedies, Medicinal plants, Malaria, Pakistan.

## 1 Introduction

Malaria is a mosquito borne disease caused by parasitic protozoa of the genus Plasmodium; *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* (1). Mortality usually results from *P. falciparum* because the other species *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria (2). *P. falciparum* is the deadliest predominate in Africa where this responsible for 90% of malarial patients death (1). While *P. vivax* is the most frequent that accounts for 65% of malaria cases in Asia and South America (Vogel, 2013). In chronic situation malaria is an important causal factor in anemia (3); (4) and has been observed with direct physical effects as lowering worker productivity and output (5), permanent neurological and cognitive damage in children (6).

Malaria is still a global health concern and endemic to 95 countries around the World (7) in tropical and sub-tropical regions including part of the America, Asia and Africa (8). It continues to be a major cause of morbidity and mortality and responsible for death of one to two million people each year (9); (10) mostly children under the age of 5. The global death due to malaria in 2015 was 4, 38,000 with most deaths occurred in the WHO African Region (88%), followed by the WHO South-East Asia Region (7). Thus, about half of the World population still at risk of malaria as 207 million cases of malaria occurred globally with 80% only in Africa (1). In order to control the havoc caused by malaria, WHO has launched malaria control program but economic burden of antimalarial drugs, insecticidal resistant anopheles and drugs resistance strains of Plasmodium are the challenges to overcome. Many researchers have called for combinations of antimalarial drugs to prevent Plasmodium spp. resistance (11) and now

artemisinin based combination therapies are efficacious against Plasmodium spp. resistance(12). Along this, strategic plans for the discovery and development of novel antimalarial compounds have transformed worldwide interest to explore plant-based antimalarial products by exploiting traditional medicinal systems(13).

Medicinal plants play a major role in many societies over the world in the treatment and prevention of disease and the promotion of general health(14). Many developing countries still depend on traditional antimalarial medicine as a source for treatment of malaria. On average, a fifth of patients use traditional medicinal plant remedies for malaria in endemic countries and above 1250 plant species from 160 families around the globe are used to treat malaria and fever(14). Investigation of new bioactive compounds from medicinal plants based on traditional use appear to be a very promising approach(15). Therefore, scientists stress on novel, cheap and effective compounds that could serve as primary antimalarial molecules. So, ethno botanical information has gained substantial attention in scientific society in arrange to set up their viability and potential as sources of modern antimalarial drugs. This survey was based on the compilation of restorative plants used in conventional medication within the treatment of intestinal sickness in Pakistan

## 2. Materials and Methods

### 2.1 Experimental Material

Pakistan is divided into five provinces; Baluchistan, Gilgit-Baltistan, Khyber Pakhtunkhwa, Punjab, Sindh, and three regions; Azad Jammu Kashmir, Federally Administered Tribal Areas and Islamabad Capital Territory. According to the United Nations estimate the population of Pakistan is 194,680,833 as of Monday, December 26, 2016, ranked 6th by population and equivalent to 2.57% of the total world population. Among these 38.6 % of the population is urban ([www.Worldometers.info](http://www.Worldometers.info)).

Pakistan is classified as a moderate malaria endemic country and with an estimated burden of 1.6 million cases annually including all age groups however; children below 5 years of age and pregnant women are at higher risk of infection (MIS report, 2013-2014). Seasonal transmission of *P. vivax* and *P. falciparum* malaria mostly occurred in the second half of the year after the July-August monsoon (16). Therefore, cases of malaria are observed with a peak around September for *P. vivax* malaria and around October for *P. falciparum* malaria. The annual par site incidence in Pakistan varies between provinces ranging from as low as (0.10) cases in Azad Jamu Kashmir (6.82) in Tribal Areas and (7.82) in Baluchistan per 1000 population. Maximum proportion of *P. falciparum* cases (44.3%) is reported from Baluchistan province followed by 27% cases in the Sindh province (MIS report, 2013-2014). Severe malaria is associated with high mortality and morbidity, especially in the young.

**2.2. Ethnomedicinal data collection:** Literature search was conducted on ethnomedicinal plants used in Pakistan for treatment of malaria and data were collected about plants use, their antiplasmodial activity and antimalarials obtained from these plants. Online databases were used such as; science direct, research gate, Google scholar and relevant journals links with specific search terms such as ethnomedicinal plants, antimalarial plants and ethnobotanical studies in Pakistan. After collection of data a master list was prepared with information like plant families name, part used and formulation method with references for each species.

## 3. Results and discussion

### 3.1 Ethnobotanical studies, diversity and distribution of antimalarial plants in Pakistan:

A number of ethnobotanical studies have been conducted in the past and many plants have been reported being used for medicinal purpose in various parts of Pakistan(17); (18);(19);(20);(21);(22);(23); S. Khan et al., 2015; S. W. Khan & Khatoon, 2008; Aqeel Mahmood & Mahmood, 2012; Mahmood et al, 2013;(24);(25);(26);(27);(28); Shah & Khan, 2006;(29);(30);(31);(32);(33);(34);(35);(36). These studies reveal that in rural areas of Pakistan, medicinal plants offers better communal acceptance, steady supply, and the prospect to support local economic activity.

These studies as focus on ethnobotanical use of plant mostly lack relevant ethnomedicinal information like formulation, dose, and time of use and toxicity of plant. Among the medicinal plants, antimalarial plants has also been documented by researchers from various parts of Pakistan, however, no research work has been conducted that only focuses on medicinal plants used against malaria from any part of Pakistan. A single review article that only highlight few medicinal plants use against Plasmodia and Leishmanial parasites from Pakistan(37). In current review, a total of (77) species of (63) genera belonging to (35) families were listed that have been traditionally used for the treatment of malaria in Pakistan (Table 1). Among the (35) families, Asteraceae contributed maximum number (18 species), followed by Lamiaceae (6 species), Gentianaceae (5 species) and Amaranthaceae (4 species). Three families Apocynaceae, Ranunculaceae and Verbenaceae each one contributed three species and six families Acanthaceae, Apiaceae,

Capparidaceae, Meliaceae, Myrtaceae, Solanaceae and Violaceae each one contributed two species. In the other families, each one contribute one species include; Asclepiadaceae, Boraginaceae, Brassicaceae, Caesalpiniaceae, Cannabinaceae, Chenopodiaceae, Euphorbiaceae, Liliaceae, Malvaceae, Menispermaceae, Oleaceae, Orchidaceae, Papilionaceae, Polygonaceae, Resedaceae, Rosaceae, Salicaceae, Sapindaceae, Valerianaceae and Zygophyllaceae. Family Asteraceae is the largest and widely distributed family and has been reported with maximum number of antimalarial species in review article from Odisha, India (38) and family Asteraceae and Lamiaceae in medicinal plants survey conducted by (39) in Boricha District, Sidama Zone, South Region of Ethiopia. Out of 5700 medicinal plants present in Pakistan, 372 medicinal plants are commonly available (40). Among these, some plants have been reported from different parts in ethnobotanical studies across Pakistan. This may link to their wide distribution and medicinal value. Among the reported antimalarial plants *Xanthium strumarium* (31), (41), (42), (20); (43), (44), (34), (26), (45), (46), (41), (47), S. Khan et al., 2013, R. Khan et al., 2013), *Calatropisprocera* (48,49), (50-52), Mahmood et al., 2011, (26), (53), (36), and (54). *Azadirachta indica* are commonly available plants and have been documented in more than five of the ethnobotanical studies conducted across Pakistan (A Mahmood et al., 2011, Marwat and Rehman, 2011, (48,55), (56), Khan et al., 2016, (57), (28). However, some plants e. g. *Artemisia* species due to their distribution in some parts of Pakistan have been documented in the ethnobotanical studies (58,59), (60), (61), (44) conducted in these areas

**Table.1 Ethnomedicinally used antimalarial plants in Pakistan.**

S/No	Family	Plants Name	Parts used	Habit	Area	Province or territory	Reference
1	Acanthaceae	<i>Barleria acanthoides</i> Oerst.	Leaves	Shrub	Thar desert	Sindh	(36)
		<i>Sticticia adhatoda</i> L.	Whole plant	Shrub	Jhelum	Punjab	(56)
2	Amaranthaceae	<i>Achyranthes aspera</i> L.	Hot decoction as emetic in malaria	Herb	Sialkot	Punjab	Mahmood et al., 2011)
		<i>Aerva javanica</i> (Burm. f.) Juss.	Flower, leaves	Herb	Bahawalpur	Punjab	(57)
		<i>Amaranthus spinosus</i> L.	Whole plant	Herb	Swabi	Khyber Pakhtunkhwa	(51)
		<i>Alternanthera pungens</i> Kunth	Whole plant	Herb	----	Khyber Pakhtunkhwa	(62)
3	Apiaceae	<i>Ferula narthex</i> Boiss.	Whole plant	Herb	Kurram	Khyber Pakhtunkhwa	Hussain et al., 2012)
		<i>Psammogeton natum</i> Edgew.	Whole plant	Herb	-----	-----	(63)
4	Apocynaceae	<i>Conium maculatum</i> L. R Br	Whole plant or inner bark is used as a source anti-malarial drug	Tree	Sargodha	Khyber Pakhtunkhwa Punjab	(64) (28)
		<i>Catharanthus roseus</i> (Linn.) G. Don	Leaves	Herb	Bahawalpur	Punjab	(57)
		<i>Albizia julibrissin</i> L.	Flower, Shoot	Shrub	Sargodha	Punjab	(28)
5	Asclepiadaceae	<i>Calatropis procera</i> (Wight) Ali	Stem, leaves, root and fruit	Shrub	Mirpur Khas City, Sialkot, Muzaffargarh, Bahawalpur, Dera Ghazi Khan, Coastal area,	Khyber Pakhtunkhwa Punjab Balochistan, FATA, Jammu and Kashmir Sindh	Mahmood et al., 2009), (49), (Mahmood et al., 2011), (Nisar et al., 2011), (53), (36), (54).

					umber, Mandi audin, Thar Desert, Chail valley Swat		
6	Asteraceae	berboa ramosa (Roxb.) Jafri	Whole plant	Herb	Nara Desert	Sindh	(65)
		Artemisia absinthium L.	Leaves	Herb	Malakand Kurram	Khyber Pakhtunkhwa, Punjab, Gilgit Baltistan ,	(Abbas et al., 10),(60),(61), (W. Hussain et al., 2012)
		Artemisia annua L.	Whole plant (Tea)	Herb	Chadori Valley	Gilgit Baltistan Azad Kashmir	(49), (Ch et al., 2013)
		Artemisia japonica Thunb	Flowers and leaves	Herb	Northern areas of Pakistan		(59)
		Artemisia maritima L. ex Hook.f.	Leaves and Stem	Shrub	Chitral Gol National Park	Khyber Pakhtunkhwa	(22)
		Artemisia moorcroftiana Wall. ex DC.	Whole plant	Herb	Northern areas of Pakistan		(59)
		Artemisia roxburghiana Vall. ex Besser	Whole plant	Herb	Northern areas of Pakistan		(59)
		Artemisia scoparia L.	Floral part	Herb	Khyber Pakhtunkhwa, Mastriata, New Murree, Gujranwala	Khyber Pakhtunkhwa	(20,66) (67)
		Artemisia vulgaris L.	Leaves	Herb	Northern areas of Pakistan		(59)
		Aster trinervius Roxb	Root	Herb	South Waziristan	Khyber Pakhtunkhwa	(68)
		Aster chinensis (L.) Willd.	Floral part	Herb	Bumburet, Balash valley, Chitral	Khyber Pakhtunkhwa	(47)
		Aster tripartita L.	Floral part	Herb	Bumburet, Balash valley, Chitral	Khyber Pakhtunkhwa	(47)
		Artemisia intybus L.	Leaves and root	Herb		Khyber Pakhtunkhwa Gilgit- Baltistan	(69); Shah & Hussain, 2012) ,(19). (B. Khan et al., 2011)
		Artemisia serriola L.	Whole plant	Herb	Jhelum	Punjab	(56)
		Parthenium sterophorus L.	Whole plant	Herb	Khyber	Khyber Pakhtunkhwa	(47)
		Seriphidium kurramensis (Qazilb.) Y.R. Ling, Bull	Leaves	Shrub	Kurram	Khyber Pakhtunkhwa	W. Hussain et al., 2013)(69)

		Vernonia perascens. Sch. Bip.	Whole plant	Herb	Bahawalpur	Punjab	(70)
		Xanthium strumarium L.	Whole plant	Herb	Battagram Bahawalpur Loralai Baluchistan, Swat, Khyber	ber Pakhtunkhwa Punjab Baluchistan, Khyber Pakhtunkhwa	(31,41), (42), (20); R. Khan et al., 2013), (W. Hussain et al., 2012), (M. Ullah et al., 2013), (Nisar et al., 2014).(45) (Akhtar et al., 2013),(71),(47)(S.( 72),
7	Boragina ceae	Heliotropium europaeum L.	Whole Plant	Herb	helum, Thar Desert	Punjab, Sindh	(56) (36)
8	Brassica ceae	Capsella bursa pastoris. (L.)Medic.	Leaves	Herb	ehsil Kabal, Swat	Khyber Pakhtunkhwa	(I.(73)
9	Caesalpi niaceae	assia fistula L.	aves, flower, seed		Mirpur	zad Jammu and Kashmir	(67,74)
10	Cannabi naceae	Cannabis sativa.Mill.	Leaves	Herb	otabad City, anga Manga orest, Leepa Valley	ber Pakhtunkhwa Punjab Northern Areas Azad Kashmir	(Begum and assumyaseen., 2013), (Sabeen & Ahmad, 9), (K. Khan et al., 2009.),(76)
11	Capparid aceae	pparis spinosa L.	Floral part	Shrub	ational Park Gilgit Khunjerab. Bumburet, Kalash ley, Chitral	kyber Pakhtunkhwa Gilgit Baltistan	(47), (B. Khan et al., 2011)
		leome scaposa DC.	Whole Plant	Herb	listan desert	Punjab	((77)
12	Chenopo diaceae	Chenopodium mbrosioides L.	Shoot	Herb	Kabal,Swat	Khyber Pakhtunkhwa	(I.(73)
13	Euphorbi aceae	Breynia cernua (r.) Muell. Arg.	Shoots	Shrub	entral Punjab	Punjab	(55)
14	Gentiana ceae	Enicostemma hyssopifolium (Willd.)Verd.	Whole Plant	Herb	Coastal area	Balochistan	(51)
		Gentianodes tianschanica (Rupr.ex Kusn.) Omer, Ali & Qaiser	Leaves	Herb	aramosh and grote Valley	Gilgit Baltistan	an & Khatoon, 2008)
		wertia chiraita (Wall.) C.B. Clarke	aves/Whole plant	Herb	atriata, New Murree, otabad City	Khyber Pakhtunkhwa, Punjab	(78) (52)

		<i>vertia cordata</i> Wall.	Leaves	Herb	atriata, New Murree	Punjab	(78)
		<i>rtia paniculata</i> Wall.	Whole Plant	Herb	Allai Valley	Khyber Pakhtunkhwa	(41)
15	Lamiaceae	<i>juga bracteosa</i> <i>llich ex Benth.</i>	Whole plant  ed leaves are rounded and form pills	Herb	Mansehra botabad City Maradori Valley, Azad Kashmir	Khyber Pakhtunkhwa Azad Kashmir	(79) (52) Ahmed et al., 2013)
		<i>cas cephalotes</i> (Roth.) Spreng.	leaves, shoot	Herb	Himalayas	Kashmir	(29)
		<i>Marrubium</i> <i>vulgare L.</i>	leaves, Root	Herb	alagai hills, Kabul valley	Khyber Pakhtunkhwa	(18)
		<i>num basilicum</i> L.	es and Seed	Herb	Rawalpindi	Punjab	(40)
		<i>lvia bucharica</i> M. Pop	Leaves and Flowers	Herb	Kalat and Khuzdar	Balochistan	(33)
		<i>Teucrium</i> <i>ksianum Boiss</i>	Whole Plant	Herb	Ziarat	Balochistan	(27)
16	Liliaceae	<i>Allium cepa L</i>	b and leaves	Herb	-----	Khyber Pakhtunkhwa	an R. U et al., 2013)
17	Malvaceae	<i>ombax ceiba L.</i>	Gum	Tree	Sargodha	Punjab	Shah et al., 2015)
18	Meliaceae	<i>dirachta indica</i> A. Juss. 1	leaves, Bark ion (about 1 ) is given in morning and ning to treat malaria	Herb	Sialkot Bahawalpur Gujrat Sargodha ntral Punjab	Khyber Pakhtunkhwa	(80)(Marwat & Rehman, 2011),(48) (55) (56), (Khan et al., 2016),(57), (A Shah et al., 2015)
		<i>a azedarach L.</i>	Seed	Tree	Sargodha	Punjab	Shah et al., 2015)
19	Menispermaceae	<i>Cissampelos</i> <i>pareria L.</i>	Whole Plant	Shrub	argalla Hills ational Park	Islamabad	(81)
20	Myrtaceae	<i>Eucalyptus</i> <i>camaldulensis</i> Dehnh.	es and Stem	Tree	Coastal area	Balochistan	(51)
		<i>Eucalyptus</i> <i>globules Labill.</i>	and Leaves	Tree	Mianwali, Gujrat	Punjab	Ahmad, 2006), (K. assain et al., 2010)
21	Oleaceae	<i>Fraxinus</i> <i>noxyloides (G.</i> Don) DC.	es and Root Bark	Shrub	rthern Areas		(23)
22	Orchidaceae	<i>Satyrium</i> <i>alense D. Don</i>	Tuber	Herb	-----	Gilgit Baltistan	(82)
23	Papilionaceae	<i>Indigofera</i> <i>argentea</i>	Whole plant	Herb	istan Desert	Punjab	Ahmad et al., 2014)
24	Polygonaceae	<i>Bupleurum</i> <i>ngicaule Wall.</i>	whole plant	Herb	ne and Sub- pine regions adori Valley	Pakistan Azad Kashmir	(21) (83)

25	Ranunculaceae	Delphinium brunonianum Royle	Leaves, Flower	Herb	Northern Areas		(23)
		Delphinium nitidum Wall. ex Royle	Root	Herb	Haromosh and Grotte valleys	Gilgit Baltistan	(An & Khatoon, 2008)
		Ranunculus arvensis L.	Whole plant	Herb	Khyber Pakhtunkhwa	Khyber Pakhtunkhwa	(62)
26	Resedaceae	Isotria medeoloides L.	Whole plant	Herb	Barban Hills, Abbottabad	Khyber Pakhtunkhwa	(84)
27	Rosaceae	Cotoneaster microphyllus Wall. ex Lindl.	Fruit	Herb	Kurram	Khyber Pakhtunkhwa	(Hussain et al., 2012)
28	Rutaceae	Xanthoxylum armatum			Cheshil Kabal, Swat	Khyber Pakhtunkhwa	(85)
29	Salicaceae	Salix acmophylla Boiss.	Leaves	Shrub	Chitral	Khyber Pakhtunkhwa	(Ali et al., 2012)
30	Sapindaceae	Albizia viscosa (L.) Jacq.	Seed	Shrub	Allai valley	Khyber Pakhtunkhwa	(41)
31	Solanaceae	Datura innoxia Mill	Leaves, seed and fruit	Herb	Rawalpindi, Thar Desert	Punjab, Sindh	(36,40)
		Datura stramonium L.	Leaves	Herb	Changa Manga Forest	Punjab	(75)
32	Valerianaceae	Valeriana jatamansi Jones	Root	Herb	Malagail hills, Kabal valley	Khyber Pakhtunkhwa	(18)
33	Verbenaaceae	Veronica camara L.	Whole plant	Herb	Chakerwal and Gula khel	Khyber Pakhtunkhwa	(62)
		Veronica officinalis L.	Whole plant	Herb	Swat, Dera Ghazi Khan	Khyber Pakhtunkhwa, Punjab	(46), (49), (H. Sher et al., 2010), (85)
		Veronica negundo L.	Leaves/Root	Tree	Malagail hills, Kabal valley	Khyber Pakhtunkhwa	(18)
34	Violaceae	Viola biflora L.	Fruit	Herb	Bumburet, Malash valley, Chitral	Khyber Pakhtunkhwa	(47)
		Viola odorata L.	Whole plant	Herb	Adori Valley	Azad Kashmir	(83)
35	Zygophyllaceae	Ziziphium harmala L.	Seed	Herb	Northern Areas, South Waziristan	Northern Areas, South Waziristan	(34,48)

### 3.3 Plant parts formulation and use

Information regarding plant part used in formulation of remedies is essential because the active chemical ingredients vary within plant parts (86); (87). All parts of the reported plants have been mentioned used for treatment of malaria including bulb, flower, fruit, leaves, root, seed, stem and tuber. Some authors have mentioned more than one parts of the same plant or whole plant used as antimalarial agent e. g. Artemisia absinthium leaves, seeds or whole plant, A. japonica stem and leaves, A. maritima leaves and stem, A. scoparia floral parts and leaves, Azadirachta indica leaves, bark and flower, Calatropis procera stem, leaves, root, bark, whole plant and flower, Capparis spinosa floral bud and fruit, Cassia fistula leaves, flower and seed, Cichorium intybus leaves and root, flower, Coriandrum sativum stem, leaves and fruit, Datura innoxia leaves, seed and fruit, Delphinium brunonianum leaves and flower, Eucalyptus camaldulensis leaves and stem, Eucalyptus globulus seed and leaves, Fraxinus xanthoxyloides leaves, root

and bark, *Leucas cephalotes* leaves and shoot, *Ocimum basilicum* leaves and seed, *Plumeria rubra* flower and shoot, *Salvia bucharica* leaves and flowers, *Swertia chiraita*, leaves and whole plant, *Vitex negundo* leaves and root; and fruit, leaves or whole plant of *Xanthium strumarium* have been reported used in synthesis of remedies (Table 1).

Among the plant parts leaves of most plants have been mentioned with relative use (34.48%). Whole plant has also been frequently reported with relative use (33.33%). However, in traditional therapies whole plant is rarely used for medicine as one part of those plants may be quite toxic or useless and another may be quite harmless and useful (88). In the other parts, root percent use was calculated (9.19%), followed by seed (8.04%), stem (5.74%), floral part (4.59%), fruit (4.59%), bulb (1.14%) and tuber (1.14%). Leaves have also been reported as frequently used plant part in review article 'ethnomedicinal plants of Odisha used for the treatment of malaria (38), Sidama People of Boricha District, Sidama Zone, South Region of Ethiopia (39), Adamawa State, Nigeria Dluya(89), Bangladesh(90) and Karnataka, India (Prakash & Payyappallimanaa 2013).

People in various parts of Pakistan practiced medicinal plants to treat ailments based on their previous knowledge and experience. Such information has been documented in the ethnobotanical studies however, many plants have been reported as antimalarial agents without supporting data like; form of remedy, dosage and time of use(84), Amin Shah et al., 2013,(55), W. Hussain et al., 2012 etc.). According to (91), improper preparations, dosage, harmful side effects and ambiguous product create potential risk if not administered appropriately. Information about form, dosage and time of use of remedies is also essential for further evaluation and safety in health management.

A few of the plants have been reported in many research articles e. g., *Xanthium strumarium*, *Calatropis procera*, *Azadirachta indica*, *Cannabis sativa* and *Artemisia absinthium* that indicate the familiarity of these plants used among various societies in Pakistan for treatment of malaria. These mostly reported plants include; *Xanthium strumarium* reported in (14) research publications followed by *Calatropis procera*, *Azadirachta indica*, *Cannabis sativa*, *Artemisia absinthium* in (10), (8), (5) and (5) research publications, respectively. In the other plants, three of the plants species; *Artemisia scoparia*, *Cichorium intybus* and *Verbena officinalis* were reported in three research publications.

### 3.4 Antiplasmodial activity of traditional medicinal plants

Pakistan is a developing and moderate malaria endemic country ([dmc.gov.pk/index.php](http://dmc.gov.pk/index.php)), where most people use the traditional medicine for the treatment of malaria. Among the reported antimalarial plants from Pakistan, several plants have been investigated phytochemically and pharmacologically across the globe for antiplasmodial activity (Table 2) and have shown good antiplasmodial activity (92)(93) et al., 2014; (94);(95);(96);(97);(98); (99);(100); (101); (102); (103); (104); (105);(106);(107);(108);(109);(110);(111);(112);(113);(114);(115);(116). As, the active constituents varies within plant species among various geographic regions and are influenced by various factors including seasons, environment, plant part, intra-species variation, and plant age(117). Therefore, further evaluation is necessary to assess their antimalarial potential, bioactive constituents and to establish their efficacy for better health opportunity.

Along this, literature search on the following plants indicates that no antiplasmodial activity has been conducted that confirm their ethnomedicinal value as used for treatment of malaria in Pakistan. These plants include; *Allium cepa* L., *Alternanthera pungens* Kunth., *Amberboa ramosa* (Roxb.) Jafri., *Artemisia japonica* Thunb., *Artemisia maritima* L. ex Hook.f., *Artemisia moorcroftiana* Wall. ex DC., *Artemisia scoparia* L., *Artemisia vulgaris* L., *Aster trinervius* Roxb., *Barleria acanthoides* Oerst., *Bidens chinensis* (L.) Willd., *Bidens tripartita* L., *Bombax ceiba* L. *Breynia cernua* (Poir.) Muell. Arg., *Bupleurum longicaule* Wall. *Cannabis sativa* Mill., *Capsella bursapastoris* (L.) Medic., *Catharanthus roseus* (Linn.) G. Don *Cleome scaposa* DC. *Cotoneaster microphyllus* Wall. ex Lindl. *Datura innoxia* Mill, *Datura stramonium* L. *Delphinium brunonianum* Royle, *Delphinium vestitum* Wall. ex Royle, *Encostemma hyssopifolium* (Willd.) Verd., *Eucalyptus camaldulensis* Dehnh. *Ferula narthex* Boiss. *Fraxinus xanthoxyloides* (G. Don) DC. *Gentianodes tianschanica* (Rupr. ex Kusn.) Omer, Ali & Qaiser, *Indigofera argentea*, *Leucas cephalotes* (Roth.) Spreng. *Marrubium vulgare* L., *Melia azedarach* L., *Parthenium hysterophorus* L., *Psammogeton biternatum* Edgew., *Ranunculus arvensis* L., *Reseda odorata* L., *Salix acmophylla* Boiss., *Salvia bucharica* M. Pop., *Satyrium nepalense* D. Don., *Seriphidium kurransensis* (Qazilb.) Y. R. Ling, Bul., *Swertia cordata* Wall., *Swertia paniculata* Wall., *Teucrium stocksianum* Boiss., *Valeriana jatamansi* Jones, *Verbena officinalis* L. and *Viola biflora* L. Therefore, evaluation of these plants is important in order to claim their antimalarial potential through experiments. On the other hand, several plants were found whose generic allies have been investigated for antiplasmodial activities and have been confirmed as potent source of antimalarials. Therefore, on chemotaxonomic basis these plants necessitate pharmacological evaluation in order to confirm their potential as antimalarial agents.

The idea that just because traditional medicine products come from natural sources they are completely safe is dangerously false (118). Now, they are examined to assess their effectiveness and to monitor their adverse effects ((119); (120)) Out of all the reported (77) antimalarial plants, no one has been mentioned as having toxic effect in the ethnobotanical studies. But literature search shows that not all of them are safe, e.g. *Datura stramonium*, *Delphinium brunonianum*, *C. procera* and *Capparis spinosa* (121); (122);(123);(124) have been investigated for their toxic effect and have been confirmed as toxic agents. According to (125) the risks

are relatively small when traditional medicines are used correctly. So, for safety, efficacy, quality, and pharmacovigilance we should recognize we should recognize the unique historical development of these products (WHO, 2004 b; WHO, 2004 a). It has gotten to be fundamental, subsequently, to outfit the common open counting healthcare experts with satisfactory data to encourage superior understanding of the dangers related to the to utilize of these items and to guarantee that all solutions are secure and of reasonable quality.

**Table.2 The list of antiplasmodial activity of traditional medicinal plants.**

Plants Name	Plant part extract/amount	% inhibition	References
Achyranthes aspera L.	Stem ethanolic extract	% inhibition	(116)
	2.80 mg dry extract / 80 mg plant dry material	$\mu\text{g/ml}$ (P. falciparum)	
	100 $\mu\text{g/ml}$	37	
	50 $\mu\text{g/ml}$	26	
	25 $\mu\text{g/ml}$	3	
	$\text{IC}_{50}$ $\mu\text{g/ml}$	153	
Achyranthes aspera L.	Root % yield	% inhibition 10	(113)
	Crude extract (8.9)	$\mu\text{g/ml}$ (P. falciparum)	
	Cyclohexane extract (1.7)	20	
	Methylene chloride extract (0.5)	39	
	Methanol extract (58.7)	0	
		10	
Achyranthes aspera L.	Ethanol extract of plant part used	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ ) (P. falciparum)	(105)
	Leaf	>100.	
	Stem	76.75	
	Root	>100	
	Seed	>100	
	Methanolic extract	$\text{IC}_{50}$ (47.18) (P. falciparum)	(92)
Adhatoda vasica Nees.(Jasticia adhatoda L.)	Leaf methanolic extract	$\text{IC}_{50}$ $\mu\text{g/ml}$ >100 (P. falciparum)	(106)
	Ethanol extract leaves Vasicine Vasicinone	( $\text{IC}_{50}$ = 89.8 $\mu\text{g/mL}$ ) (P. falciparum)	(102)

	VA-1	(IC <sub>50</sub> = 38.9 μg/mL)  ( IC <sub>50</sub> = 06.0 μg/mL)	
<i>Aerva javanica</i> (Burm. f.) Juss. ex Schult.	Fruit ethanolic extract  4mg dry extract / 80mg plant dry material  100 μg/ml  50 μg/ml  25 μg/ml  12.5 μg/ml  IC <sub>50</sub> μg/ml  Stem ethanolic extract  3 mg dry extract / 80mg plant dry material  100 μg/ml  50 μg/ml  25 μg/ml  IC <sub>50</sub> μg/ml	( <i>P. falciparum</i> )  60 36 21 9 76  35 25 17 308	(116)
<i>Ajuga bracteosa</i> Wallich ex Benth.	Leaves ethanolic extract  750 mg/kg/day maximum	77.7% chemo suppression during early infection and 68.8% chemo suppression in repository activity ( <i>Plasmodium berghei</i> ).	(96)
<i>Alstonia scholaris</i> L.R Br	Bark methanolic extract  Dose-dependent	Improvement of conditions and delayed mortality amongst animals ( <i>Plasmodium berghei</i> )	(101)
<i>Amaranthus spinosus</i> L.	Water (stem bark)	789.36 ± 7.19	(104)
<i>Artemisia absinthium</i> L.	Leaves 2.00 mg/ml  Aqueous  Cold alcoholic	( <i>P. falciparum</i> )  35%  55%	(127)



	Leaves EC50 or per cent cell growth (at conc. g/ml) H <sub>2</sub> O H <sub>2</sub> O–MeOH MeOH	( <i>P. falciparum</i> ) 78% (7.0) 73% (6.5) 5.6	(107)
	Leaves ethanolic extract mg/kg 500 750 1000	Inhibited parasitaemia ( <i>Plasmodium berghei</i> ) 79.3 79.6 87.3%	(129)
Azadirachta indica A. Juss.	Leaves	Ethanol: 2.50 (FcB1) Ethanol: 2.40 (F32) <i>Plasmodium falciparum</i> : FcB1-Colombia (Chloroquine-resistant) and F32-Tanzania (Chloroquine-sensitive)	(95)
	Root dichloromethane extract mg/kg/day 200 400 800	Suppressed parasites growth in vivo by  20.70% 35.3% 55.96%	(114)
	Young twigs and leaves  Ethyl acetate Petroleum ether	Chloroquine sensitive strain of ( <i>P. falciparum</i> ) against FCR-3 EC50 16 µg/mL 18 µg/mL	(115)
	Seeds extract at concentration 2.00 mg/ml Aqueous Cold alcoholic Hot alcoholic	( <i>P. falciparum</i> )  65  56  76	(127)



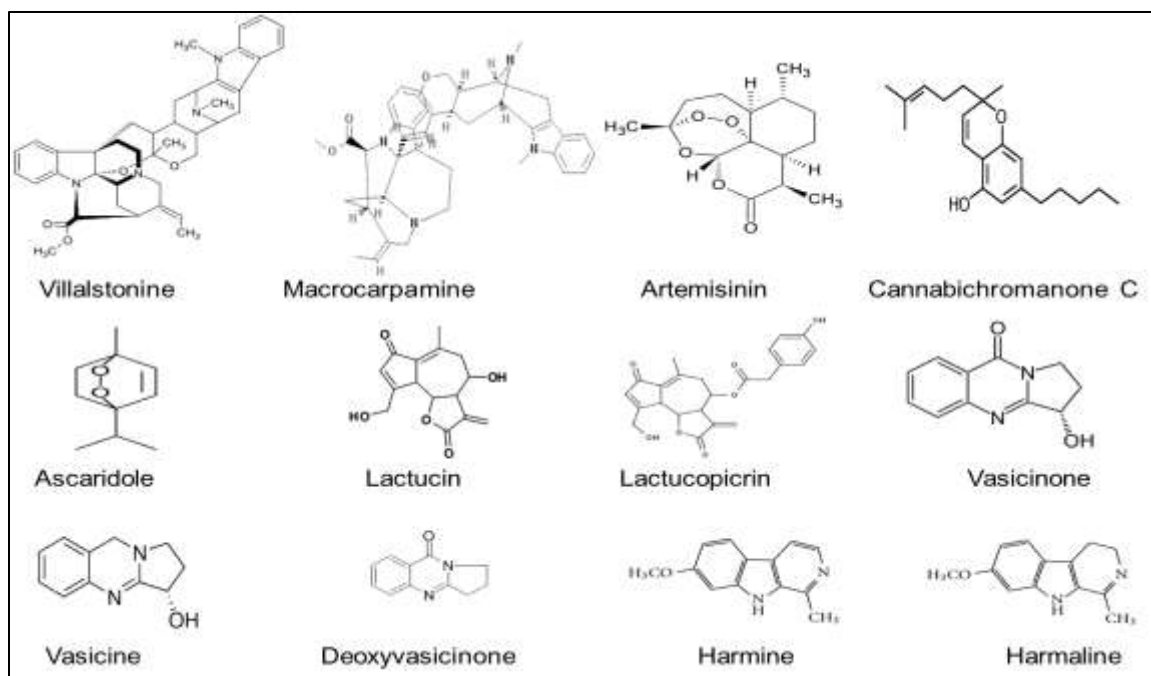
Chenopodium ambrosioides L.	Leaves hydroalcoholic extract (HCE)	HCE could bind to the total proteins of infected erythrocytes and could inhibit the parasite growth in vitro (IC <sub>50</sub> = 25.4 g/mL). The in vivo therapeutic treatment with HCE increased the survival and decreased the parasitaemia in the infected animals	(98)
Cichoriun intybus L.	Methanolic extract	IC <sub>50</sub> >64 (P. falciparum)	(Abdel-Sattar et al., 2010)
Cissampelos pareria L.	Ethanollic root extract Mean parasitaemia of untreated group Mean parasitaemia of extract treated group	(Plasmodium berghei) 25.48 ± 4.88 11.64 ± 4.20%	(130)
Dodonaea viscosa (L.) Jacq.	Fruit ethanolic extract 20.60 mg dry extract / 80 mg plant dry material  Leaf ethanolic extract 14.50mg dry extract / 80mg plant dry material  Stem ethanolic extract 4mg dry extract / 80mg plant dry material	% inhibition µg/ml (P. falciparum) 100µg/ml = 99 50µg/ml = 90 25µg/ml = 63 12.5µg/ml = 39 IC <sub>50</sub> µg/ml = 16  100 µg/ml = 98 50 µg/ml = 94 25µg/ml = 55 12.5µg/ml = 26 IC <sub>50</sub> µg/ml = 22  100 µg/ml = 93 50 µg/ml = 69 25µg/ml = 37 12.5µg/ml = 10 IC <sub>50</sub> µg/ml = 34	(116)
Eucalyptus globulus Labill	Strain Fc81 (Chloroquine resistant) Nigerian (Chloroquine sensitive)	Plant 24 h 72 h 24 h 72 h (P. falciparum)  >1000 > 1000 >1000 750	(111)

Heliotropium europaeum Linn.	Flower 4.70 mg (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5µg) Root 1.70 mg (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5µg) Stem 7.60 mg (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5µg)	(30 10 6 -3) IC <sub>50</sub> 395 (P. falciparum) (91 59 19 -6) IC <sub>50</sub> 43 (80 72 37 13) IC <sub>50</sub> 35	(116)
Jasticia adhatoda L. (Adhatoda vasica Nees.)	Leaf methanolic extract	IC <sub>50</sub> µg/ml >100 (P. falciparum)	(106)
Lactuca serriola L.	Methanolic extract	IC <sub>50</sub> >64 (P. falciparum)	(92)
Lantana camara L.	Aqueous and ethanolic extracts were 38 and 76% effective respectively.  Antiprotozoal activity IC <sub>50</sub> (µg/ml) 12.0±2.2  Leaves, dichloromethane	(P. falciparum)  8.7 ± 1.0 3D7 S 5.7 ± 1.6	(100) (110)
Ocimum basilicum L.	Leaf ethanolic extract  4.50mg dry extract / 80 mg plant dry material  Stem ethanolic extract  2.50mg dry extract / 80 mg plant dry material	% inhibition µg/ml (P. falciparum) 100 µg/ml = 64 50 µg/ml = 28 25µg/ml = 14 12.5 µg/ml = 8 IC <sub>50</sub> µg/ml = 81  % inhibition µg/ml 100 µg/ml = 39 50 µg/ml = 22 25µg/ml = 9 12.5µg/ml = -11 IC <sub>50</sub> µg/ml = 157	(116)
Peganum harmala L.	Wood ethanolic extract  8.30mg dry extract / 80 mg plant dry material	% inhibition µg/ml(P. falciparum) 100 µg/ml = 95 50 µg/ml = 79 25µg/ml = 29 12.5µg/ml = 4 IC <sub>50</sub> µg/ml = 34	(116)
Plumeria rubra L	Dry leaves and stem methanolic extract proved most potent in	(P. falciparum)	(Mathewet al., 2016)



	artemisinin artemetin casticin chrysopenetin chrysosplenol-D cirsilineol eupatorin	--- 2.6 2.4 2.3 3.2 3.6 6.5	3.3 2.6 2.6 2.25 1.5 1.6 3.0	
Cannabis sativa L.	6-pre-nylapigenin	IC50 values D6 strain 2.7 µg/ml W2 strain 2.4 µg/ml		(133)
	Cannabichromanone A derivative  Cannabichromanone C	IC50 values D6 strain 11.1 µM W2 strain 11.4 µM  D6 strain 13.1 µMSS W2 strain 9.4 µM		(132)
Chenopodium ambrosioides L.	Ascaridole	Strong antimalarial (Plasmodium falciparum) Development was arrested by a drug concentration of 0.05 µM, and at 0.1 µM no parasites were visible in the culture.		(136)
Cichorium intybus L.	Lactucin, Lactucopicrin	HB3 clone of strain Honduras-1 of Plasmodium falciparum, (Chloroquine sensitive and pyrimethamine Resistant).  Complete inhibition at; 10 µg/ml 50 µg/ml		(134)
Peganum harmala L.	Harmine Harmaline	37.7 (Plasmodium falciparum) >50		(94)

### 3.6 Structural configuration of antimalarial compounds



### 3.7 Clinical investigation and preparation of herbal remedies

As limited information have been recorded in the ethnobotanical studies about the antimalarial plant from various parts of Pakistan, mostly without method of formulation of remedies, dosage, time of use, duration, quality etc., Such information is required to investigate their therapeutic potential. Clinical investigation after determination of antiplasmodial activity is necessary in order to prepare novel antimalarial herbal remedies. Unfortunately, in our country Pakistan there is no such incentive due to limited standardized research facilities. Thus, evaluation of these natural remedies may provide better opportunities for formulation of remedies and confirming their safety.

### 4. Conclusion

The ethnobotanical studies carried out in Pakistan have allowed the description of a large number of plants used by people to treat different diseases, especially malaria in the moderate endemic areas. A total of (77) plants belonging to (63) genera and (35) families were identified, which are being used for the treatment of malaria. From the review it is concluded that these medicinal plants have been used over history for treatment of malaria but evaluation of their pharmacological activities is essential in order to establish their mode of action, safety, quality, efficacy and to find novel antimalarial and to evolve new drugs for resistance of Plasmodium species.

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