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IN VITRO AND IN VIVO ANTIMALARIAL AND CYTOTOXIC ACTIVITY OF FIVE PLANTS USED IN CONGOLESE TRADITIONAL MEDECINE

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Abstract

Aim of the study

The *in vitro* antiplasmodial activity and cytotoxicity of methanolic and dichloromethane extracts from five Congolese plants were evaluated. The plants were selected following an ethnobotanical survey conducted in D.R. Congo and focusing on plants used traditionally to treat malaria. The *in vivo* antimalarial activity of aqueous and methanolic extracts active *in vitro* was also determined in mice infected by *Plasmodium berghei berghei*.

Materials and methods

The growth inhibition of *Plasmodium falciparum* strains was evaluated using the measurement of lactate dehydrogenase activity. The extracts (aqueous, CH_3OH, EtOH and CH_2Cl_2) were prepared by maceration and tested *in vitro* against the 3D7 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum* and against the human normal fetal lung fibroblasts WI-38 to determine the selectivity index. Some extracts were also used at the dose of 300mg/kg to evaluate their activity in mice infected since 4 days by *Plasmodium berghe*i.

Results

Two plants presented a very high activity (IC_{50} < 3µg/ml). These plants were *Strychnos icaja* roots bark (MeOH and CH_2Cl_2) and *Physalis angulata* leaves (MeOH and CH_2Cl_2). One plant (*Anisopappus chinensis* whole plant, MeOH and CH_2Cl_2) presented a high activity (IC50 < 15µg/ml). The extracts of *Anisopappus chinensis* and *Physalis angulata* showed also a good inhibition of parasitemia *in vivo*. Flavonoids, phenolic acids and terpenes were identified in these plants by a general phytochemical screening method.

Conclusion

Three plants showed a very interesting antiplasmodial activity (*Anisopappus chinensis*, *Physalis angulata*, S*trychnos icaja*) and one of them showed a good selectivity index (>10, *Anisopappus chinensis*). *Anisopappus chinensis* and *Physalis angulata* were also active *in vivo*.

Key words:

Anisopappus chinensis; Entandrophragma palustre; Melia azedarach; Physalis angulata; Strychnos icaja, antiplasmodial activity, human fibroblast

1. Introduction

Malaria is still a major public health problem, especially in tropical and sub-tropical regions. It is estimated that half of the world population is still at risk of contracting malaria and an estimated 243 million cases led to nearly 863 000 deaths in 2008, mostly of African children aged below 5 years, who are the most susceptible to this disease. In Sub-Saharan regions, 45 countries were endemic for malaria in 2008 (WHO, 2009).

Democratic Republic of Congo is a tropical country located in central Africa where malaria with *Plasmodium falciparum* is highly endemic, being one of the most important health problems in the country. Since the 1960s, the spread of resistance to most antimalarials (choroquine, pyrimethamine, sulfadoxine, mefloquine, quinine) led to an evident need for new anti-malarial drugs, and medicinal plants constitute a reliable source of these, particularly in D.R. Congo where several plants are used alone or in combination by the population to treat malaria or other diseases. D.R.Congo is one of the richest countries in natural resources and the diversity of its botanicals resources is particularly important. Many medicinals plants growing in this area are not yet investigated. Furthermore, the ACT recommended in D.R.Congo is artesunate-amodiaquine, but the use of this drug is very limited in some rural areas where the population prefer traditional, less expensive preparations (WHO, 2009).

Previously, a research program has been initiated by some of the authors for the evaluation of the antiamoebic and antiplasmodial activity of medicinal plants species traditionally used to treat amoebias and malaria in Congolese traditional medicine (Tona et al., 1998; Tona et al., 2000; Tona et al., 1999). Relying on this ethnobotanical survey conducted in D.R.Congo in 2000-2002, we selected five plants used in traditional medicine for treating malaria, fever, inflammation, amoebics, thyphoid fever… These plants were: *Anisopappus chinensis* Hook. & Arn. (Asteraceae), *Entandrophragma palustre* Staner (Meliaceae); *Melia azedarach L*. (Meliaceae), *Physalis angulata* L. (Solanaceae), *Strychnos icaja* Baill. (Loganiaceae). The present study investigates the *in vitro* antiplasmodial activity of methanolic, aqueous and dichloromethane extracts of these plants against 3D7 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum* and the in *vitro* cytotoxicity of the same extracts against the human lungs fibroblasts WI-38. The more active *in vitro* plants were then selected for *in vivo* evaluation: aqueous and in some cases methanolic, ethanolic and dichloromethane extracts were tested against *Plasmodium berghei berghei.*

2. Material and methods

2.1. Plant material

Plant samples were collected in different regions of D.R.Congo (**Table 1).** They were identified by Mr N. Nlandu of the "Institut National d'Etudes et de Recherches en Agronomie" (INERA) from the University of Kinshasa. A voucher specimen for each plant was deposited in the herbarium of the institute and for two of them in the National Botanical Garden of Belgium (Meise, Belgium). All samples collected were air-dried at room temperature with no direct sunlight for three days.

2.2. Preparation of extracts

Crude aqueous, methanolic and dichloromethane extracts were obtained by maceration of 4 g of each powdered plant sample three times in 30 ml of solvent, for 30 min under constant shaking at room temperature. For each solvent, a new plant sample was used separately. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C with a rotatory evaporator. All extracts obtained were weighed and their yield calculated. Yields were respectively: 14.1% and 2.3% (*A. chinensis* MeOH and CH_2Cl_2); 59.0% and 4.4% (*E. palustre* MeOH and CH_2Cl_2); 14.4% and 5.7% *(M. azedarach* MeOH and CH_2Cl_2); 22.6% and4.5% (*P. angulata* MeOH and CH_2Cl_2); 16.6% and 4.9% (*S. icaja* MeOH and CH_2Cl_2).

2.3. Phytochemical screening:

Alkaloids, flavonoids, phenolic acids, terpenes and tannins were identified by TLC using a standard lab protocol (Wagner and Bladt, 1984).

2.4. In vitro antiplasmodial assays

The culture of *Plasmodium falciparum* strains was carried out as previously described method (Frédérich et al., 2001). All crude extracts were evaluated *in vitro* for their activity against a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7) and the most active extracts were also evaluated against a chloroquine-resistant strain (W2). For each crude extract, a series of 8 threefold dilutions (from 200 to 0.09 µg/ml) was prepared, placed in 2 rows of a 96-well microplate and tested in triplicate. Artemisinin (98%, Sigma-Aldrich) and Chloroquine diphosphate salt (Sigma-Aldrich) were used as standards, and infected and uninfected erythrocytes were added as positive and negative controls respectively. After 48 hours of incubation at 37 ºC, the level of parasitaemia was estimated by measuring lactate dehydrogenase activity, as previously described (Jonville et al., 2008). The results were

expressed as the mean IC_{50} (the concentration of a drug that reduced the level of parasitaemia to 50%).

2.5. In vitro cytotoxic assay

Cells from the human normal foetal lung fibroblast cell line, WI-38, were cultivated *in vitro* in DMEM: Dubecco's Modified Eagle's Medium (Lonzo, Belgium), which contains 5% of L-glutamate (Lonzo, Belgium), 5% of penicillin-streptomycin (Lonzo, Belgium) and 10% of heat inactivated foetal bovine serum (Lonzo, Belgium). Then, the cells were incubated at 37 ºC in a humidified atmosphere with 5% CO_2. For each sample, 6 threefold dilutions (from 200 to 0.82 µg/ml) were prepared, placed in 3 rows of a 96-well microplate and tested at least twice. Camptothecin (Sigma) was used as a positive control. After 48 hours incubation, cell viability was determined by measuring the fibroblast mitochondrial enzyme activity, as previously described (Stevigny et al., 2002). The results were expressed by the mean of IC_{50}s of at least 2 independent assays and the selectivity index (the ratio between the cytotoxic (WI-38 cells) and antiparasitic (3D7 strain) activity) was calculated.

2.6. In vivo antimalarial activity

The *in vivo* antimalarial activity was determined using NMRI mice bred in the pet shop of the "Institut National de Recherches Biomédicales de Kinshasa (INRB)" with average weight of 23.9 gr ± 4.2. Mice were infected by the rodent parasite *Plasmodium berghei berghei* (origin: Antwerp Tropical Medicine Institute) during 4 days (D1-D4). The treatment (oral) began at day 5 by using 300mg/kg body weight of each extract in solution in saline and was delivered during 4 days (D5-D8). Parasitaemia was determined at D8 and D9. Chloroquine (25 mg/kg) and quinine (20 mg/kg) were used as positive controls; saline was used as negative control. The method was previously described (Tona et al., 2001). Ethanolic extracts were prepared as previously described for other extracts.

2.7. Statistics

Differences between control and samples in the *in vivo* assay were compared by using the student's T-test. Statistical significance was fixed at $p \leq 0.05$.

3. Results and discussion

According to WHO guidelines and previous results from our team (Jonville et al., 2008; Pink et al., 2005), antiplasmodial activity of extracts was classified as follows: highly active

extracts with IC_{50} < 5 µg/ml, promising activity at 5-15 µg/ml, moderate activity at 15-50 µg/ml and inactivity at > 50µg/ml. Among the five plants evaluated, three showed a significant antiplasmodial activity with an IC_{50} < 15µg/ml for their dichloromethane and methanolic extracts against the two strains of *Plasmodium falciparum* (*A. chinensis, P. angulata and S. icaja*), and two were highly active. *E. palustre* showed a moderate activity against the two strains of *Plasmodium* and *M. azedarach* showed a moderate activity against the two strains only for his dichloromethane extract **(Table 2**). The extracts with at least a promising level of activity (IC_{50} < 15 µg/ml) were then assessed for their cytotoxic activity in order to determine the selectivity index (see **Table 2**). The cytotoxic assay was performed on human lung fibroblasts (WI38). The SI is defined as the ratio of the cytotoxic IC_{50} value and the parasitic IC_{50} value. The cytotoxicity of several alkaloids from *Strychnos icaja* and the cytotoxicity of *Physalis angulata* extract were previously described and showed, in this last case, a SI between 4 and 5 (Frederich et al., 2000; Frederich et al., 2001; Zirihi et al., 2005). *Anisopappus chinensis* showed the higher SI for its methanolic and dichloromethane extracts, with values superior to 10. Aqueous extracts of active plants were then evaluated *in vitro, P. angulata* revealing a moderate activity and *A. chinensis* a very slight activity (> 50 µg/ml). The active extracts were finally evaluated for their *in vivo* activity, except for *S. icaja*, which contains strychnine and which was previously evaluated *in vivo* (Philippe et al., 2007). Aqueous extracts of active plants were first evaluated *in vivo*, as it is the preparation method used traditionally. The dose of 300 mg/kg was chosen according to WHO guidelines and previous lab experience (Fidock et al., 2004). As the aqueous extract of *Physalis angulata* caused a very significant inhibition of parasite growth (> 80 %), its methanolic, ethanolic and dichloromethanic extracts were also evaluated (**Table 3**). The active plants contained alkaloids, flavonoids, phenolic acids, terpenes, tannins (**Table 4**).

3.1. *Anisopappus chinensis* Hook. & Arn. (Asteraceae),

This savannah herb was harvested in Kolwezi, province of Katanga. Traditionally, an aqueous decoction of the whole plants or of the leaves is used to cure malaria, inflammation, thyphoid, pain and ulcer wounds. This work is the first report about the in *vitro* and *in vivo* antimalarial and cytotoxic activity of this plant which presented a promising activity, a relatively high selectivity index (> 10) and a very high activity *in vivo* including for the aqueous extract. The phytochemistry of this plant is completely unexplored.

3.2. *Entandrophragma palustre* Staner (Meliaceae)

This forest and savannah tree was collected in the provinces Bas-Congo and Kasaï where an aqueous decoction of leaves or stem bark is used to treat malaria, inflammation, rheumatism, stomachache and otitis. This work reports for the first time the moderate antiplasmodial and significant antimalarial

(especially at D9) activity of this plant. The only phytochemical study conducted on this plant was realized in 1934 and showed the presence of catechol and tannins (Tihon, 1934).

3.3. *Melia azedarach* L. (Meliaceae),

This shrub was collected in Maniema, in Bas-Congo provinces where an aqueous decoction of the leaves is used to cure malaria and thyphoid fever. In Asia , the extracts of fruits, leaves, stem are used to cure filarisis, the extract of fruits is used as an antifungal and insecticide, the extract of leaves is used as an antiviral and the extract of flowers as an antibacterial of the skin; in Kenya, the plant is used as an antimalarial (Muregi et al., 2004; Saleem et al., 2008; Vishnukanta Rana, 2008). The antimalarial activity against a chloroquine sensitive strain was previously reported (Ofulla et al., 1995). In this study, a moderate activity for the dichloromethane extract and a weak activity for the methanolic extract was shown against two strains of *P. falciparum* (19 and 28 µg/ml and 55 / 46 µg/ml, respectively). This plant was also active against the malaria vector *Anopheles stephensi* (Nathan et al., 2006). This plant was the subject of several publications, particularly concerning its insecticidal and pesticide properties. These properties were attributed to the presence of limonoids such as azadirachtin (Klocke et al., 1991).

3.4. *Physalis angulata* L. (Solanaceae),

This herb is a food plant in D.R.Congo and an aqueous decoction of leaves or whole plant is used to cure malaria and inflammation. It is used to treat diabetes, hepatitis, asthma and malaria in Taiwan (Hsieh et al., 2006). *Physalis angulata* showed the stronger activity (with *S. icaja*) against the two strains of *Plasmodium falciparum* (< 5µg/ml for all extracts), a SI between 4 and 5, and its aqueous extract was also active *in vivo*. The *in vitro* antimalarial activity and the cytotxicity of this plant were previously reported (Hsieh et al., 2006; Kvist et al., 2006; Zirihi et al., 2005) but we obtained a better activity in the present study. The plant is also part of a four-plants decoction used in Ghana to treat malaria and active on rats (Ankrah et al., 2003). The compounds responsible for the activity are not yet known. Some limonoids were nevertheless shown to be active against leishmaniasis (Guimaraes et al., 2009). Other known compounds from *P. angulata* include whithanolides, withangulatins (steroidal lactones) and physalins (unusual steroids) (Damu et al., 2007). Some of these compounds are cytotoxic.

3.5. *Strychnos icaja* Baill.(Loganiaceae)

This forest shrub was found in the equatorial forest and is mainly used as arrow poison (Philippe et al., 2004) but it is also used by pygmies in Cameroon to treat malaria (Neuwinger, 1996). This plant is well known in our lab and old samples, collected 50 years ago (Denoel et al., 1953), have been

studied for antiplasmodial properties. Several bisindole alkaloids were isolated from this plant and showed a high antiplasmodial and antimalarial activity (Frederich et al., 2000; Frederich et al., 2001; Frederich et al., 1999; Philippe et al., 2007). This study confirmed the activity of this plant on fresh samples.

4. Conlusions

For the first time, a promising antiplasmodial and antimalarial activity with an interesting selectivity index was demonstrated for *A.chinensis.* This plant is particularly interesting for a further investigation as very few is known about its phytochemical composition. The antiplasmodial activities of *P. angulata* and *S. icaja,* previously known, were confirmed against two strains of *Plasmodium falciparum* . A moderate antiplasmodial and antimalarial activity was also identified for the first time for *E. palustre.*

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References

Ankrah, N.A., Nyarko, A.K., Addo, P.G.A., Ofosuhene, M., Dzokoto, C., Marley, E., Addae, M.M., Ekuban, F.A., 2003. Evaluation of efficacy and safety of a herbal medicine used for the treatment of malaria. Phytotherapy Research 17, 697-701.

Damu, A.G., Kuo, P.C., Su, C.R., Kuo, T.H., Chen, T.H., Bastow, K.F., Lee, K.H., Wu, T.S., 2007. Isolation, structures, and structure-cytotoxic activity relationships of withanolides and physalins from *Physalis angulata*. Journal of Natural Products 70, 1146-1152.

Denoel, A., Jaminet, F., Detilleux, G., Merveille, L., 1953. Contribution à l'étude chimique des *Strychnos* du Congo belge. Ministère des Colonies, Direction de l'agriculture, Bruxelles.

Fidock, D.A., Rosenthal, P.J., Croft, S.L., Brun, R., Nwaka, S., 2004. Antimalarial drug discovery: efficacy models for compound screening. Nature Reviews.Drug Discovery 3, 509-520.

Frederich, M., De Pauw, M.C., Llabres, G., Tits, M., Hayette, M.P., Brandt, V., Penelle, J., De Mol, P., Angenot, L., 2000. New antimalarial and cytotoxic sungucine derivatives from *Strychnos icaja* roots. Planta Medica 66, 262-269.

Frederich, M., De Pauw, M.C., Prosperi, C., Tits, M., Brandt, V., Penelle, J., Hayette, M.P., DeMol, P., Angenot, L., 2001. Strychnogucines A and B, two new antiplasmodial bisindole alkaloids from *Strychnos icaja*. Journal of Natural Products 64, 12-16.

Frederich, M., Hayette, M.P., Tits, M., De Mol, P., Angenot, L., 1999. In vitro activities of *Strychnos* alkaloids and extracts against Plasmodium falciparum. Antimicrobial Agents and Chemotherapy 43, 2328-2331.

Guimaraes, E.T., Lima, M.S., Santos, L.A., Ribeiro, I.M., Tomassini, T.B.C., dos Santos, R.R., dos Santos, W.L.C., Soares, M.B.P., 2009. Activity of physalins purified from *Physalis angulata* in *in vitro* and *in vivo* models of cutaneous leishmaniasis. Journal of Antimicrobial Chemotherapy 64, 84-87.

Hsieh, W.T., Huang, K.Y., Lin, H.Y., Chung, J.G., 2006. *Physalis angulata* induced G2/M phase arrest in human breast cancer cells. Food and Chemical Toxicology 44, 974-983.

Jonville, M.C., Kodja, H., Humeau, L., Fournel, J., De Mol, P., Cao, M., Angenot, L., Frederich, M., 2008. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. Journal of Ethnopharmacology 120, 382-386.

Klocke, J., A., Lee, S.M., Yamasaki, R., B. , 1991. Azadirachtin derivative insecticides. U.S. patent, N° USXXAM US 5047242 A 19910910.

Kvist, L.R., Christensen, S.B., Rasmussen, H.B., Mejia, K., Gonzalez, A., 2006. Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. Journal of Ethnopharmacology 106, 390-402.

Muregi, F.W., Chhabra, S.C., Njagi, E.N.M., Lang'at-Thoruwa, C.C., Njue, W.M., Orago, A.S.S., Omar, S.A., Ndiege, I.O., 2004. Anti-plasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquine. Phytotherapy Research 18, 379-384.

Nathan, S.S., Savitha, G., George, D.K., Narmadha, A., Suganya, L., Chung, P.G., 2006. Efficacy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Bioresource Technology 97, 1316-1323.

Neuwinger, H.D., 1996. African ethnobotany: Poisons and drugs, chemistry, pharmacology, toxicology. Chapman & Hall, London.

Ofulla, A.V., Chege, G.M., Rukunga, G.M., Kiarie, F.K., Githure, J.I., Kofi-Tsekpo, M.W., 1995. In vitro antimalarial activity of extracts of *Albizia gummifera*, *Aspilia mossambicensis*, *Melia azedarach* and *Azadirachta indica* against *Plasmodium falciparum*. African Journal of Health Science 2, 309-311.

Philippe, G., Angenot, L., Tits, M., Frederich, M., 2004. About the toxicity of some *Strychnos* species and their alkaloids. Toxicon 44, 405-416.

Philippe, G., De Mol, P., Angenot, L., Tits, M., Frederich, M., 2007. In vivo antimalarial activity of isosungucine, an inolomonoterpenic alkaloid from *Strychnos icaja*. Planta Medica 73 478-479.

Pink, R., Hudson, A., Mouries, M.A., Bendig, M., 2005. Opportunities and challenges in antiparasitic drug discovery. Nature Reviews.Drug Discovery 4, 727-740.

Saleem, R., Rani, R., Ahmed, M., Sadaf, F., Ahmad, S.I., ul Zafar, N., Khan, S.S., Siddiqui, B.S., Lubna, Ansari, F., Khan, S.A., Faizi, S., 2008. Effect of cream containing *Melia azedarach* flowers on skin diseases in children. Phytomedicine 15, 231-236.

Stevigny, C., Block, S., De Pauw-Gillet, M.C., de Hoffmann, E., Llabres, G., Adjakidje, V., Quetin-Leclercq, J., 2002. Cytotoxic aporphine alkaloids from *Cassytha filiformis*. Planta Medica 68, 1042-1044.

Tihon, L., 1934. *Entandrophragma palustris* Staner. Bulletin Agricole du Congo 25, 21-25.
Tona, L., Kambu, K., Ngimbi, N., Cimanga, K., Vlietinck, A.J., 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology 61, 57-65.

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne, T., Apers, S., Totte, J., Pieters, L., Vlietinck, A.J., 2000. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. Phytomedicine 7, 31-38.

Tona, L., Mesia, K., Ngimbi, N.P., Chrimwami, B., Okond'Ahoka, Cimanga, K., De Bruyne, T., Apers, S., Hermans, N., Totte, J., Pieters, L., Vlietinck, A.J., 2001. In-vivo antimalarial activity of Cassia occidentalis, Morinda morindoides and Phyllanthus niruri. Annals of Tropical Medicine and Parasitology 95, 47-57.

Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totte, J., Vlietinck, A.J., 1999. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. Journal of Ethnopharmacology 68, 193-203.

Vishnukanta Rana, A.C., 2008. Melia azedarach: a phytopharmacological review. Pharmacognosy Reviews 2, 173-184.

Wagner, H., Bladt, S., 1984. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Springer, New-York.

WHO, 2009. World Malaria Report 2009, Visiting time: 07 january 2010

http://www.who.int/malaria/world_malaria_report_2009/en/

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B., Grellier, P., 2005. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. Journal of Ethnopharmacology 98, 281-285.

Table 1 . List of selected plants, family, vernacular names, voucher number and specific parts used.

Species		**Family**	**Vernacular name**	**Plant part**	**Collection date**	**Harvest place**	**Voucher Number Meise**	**Voucher number Kinshasa**	**Aspect**
Anisopappus chinensis	Hook. & Arn	Asteraceae	kasol sol	whole plant	Febr-08	Kolwezi	00000692238	3278	herb
Entandrophragma palustre	Staner	Meliaceae	pake	stem bark	Oct-00	Kasai	-	9287	tree
Melia azedarach	L.	Meliaceae	kamunara	leaves	Sept-07	Maniema	-	141	shrub
Physalis angulata	L.	Solanaceae	ndimba, lundumba	leaves	Febr-08	Bas-Congo	00000692869	921974	herb
Strychnos icaja	Baill.	Loganiaceae	mbondo bololo	root bark	Febr-08	Equateur	-	357	shrub

Table 2

Selectivity index (SI), *in vitro* IC_{50} values against *Plasmodium falciparum* (3D7and W2) and against WI-38 cells.

Species or compound	**Plant part**	**Extracts**	**3D7, IC_{50} µg/ml**	**W2, IC_{50} µg/ml**	**WI- 38**	**SI**
A. chinensis	whole plant	MeOH	8.82 ± 2.83	12.24 ± 1.91	126.33 ± 6.50	10.32
		CH_2Cl_2	6.53 ± 1.33	6.37 ± 1.16	98.25 ± 19.32	15.42
		H2O	76.51±23.65	nd	nd	nd
E. palustre	Stem bark	MeOH	15.84 ± 1,19	35.98 ± 4.61	nd	nd
		CH_2Cl_2	17.69 ± 2.16	23.18 ± 8.10	nd	nd
		H2O	>100	nd	nd	nd
M. azedarach	leaves	MeOH	55.13 ± 10.67	44.62 ± 3.22	nd	nd
		CH_2Cl_2	19.14 ± 10.15	28.10 ± 1.20	nd	nd
P. angulata	whole plant	MeOH	1.27 ± 0.25	3.02 ± 2.10	15.68 ± 1.11	5
		CH_2Cl_2	1.96 ± 1.21	2.00 ± 0.08	7.84 ± 0.58	4
		H2O	23.10±1.18	nd	nd	nd
S. icaja	root bark	MeOH	0.69 ± 0.25	0.42 ± 0.22	nd	nd
		CH_2Cl_2	0.84 ± 0.05	0.61 ± 0.35	nd	nd
Chloroquine	-	-	0.0073±0.0022	0.0036±0.0006	nd	nd
Artemisinin	-	-	0.0024±0.00013	0.00172±0.00043	nd	Nd
camptothecin	-	-	nd	nd	0.0192±0.020	nd

Table 3 : *In vivo* antimalarial activity (parasite growth inhibition) of extracts (300 mg/kg, oral), chloroquine (25 mg/kg, oral) and quinine (20 mg/kg, oral) on mice infected by *Plasmodium berghei berghei*.

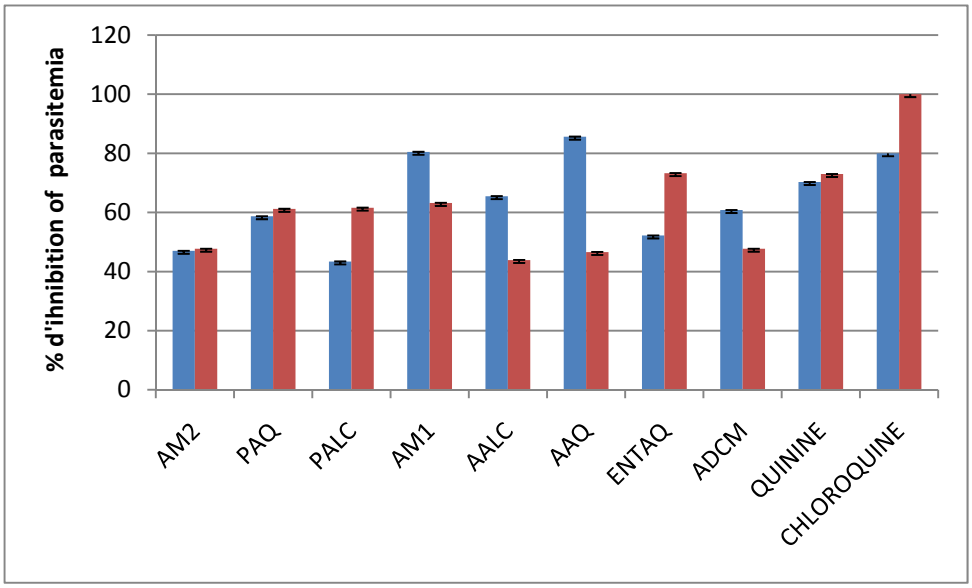
Plants	**Extracts or compound**	**Abbreviation**	**% growth inhibition at D8**	***p* value (D8)**	**% growth inhibition at D9**	***p* value (D9)**
P. angulata	aqueous	PAQ	58.7	0.005	61.2	0.006
P. angulata	ethanolic	PALC	43.4	0.005	61.6	0.010
A. chinensis	methanolic	AM1	80.5	0.001	63.2	0.007
A. chinensis	ethanolic	AALC	65.5	0.004	43.9	0.047
A. chinensis	dichloromethane	ADCM	60.8	0.013	47.7	0.035
A. chinensis	aqueous	AAQ	85.6	0.002	46.6	0.027
E. palustre	aqueous	ENTAQ	52.2	0.036	73.3	0.034
Pos. Control	Chloroquine		80.0	0.002	100.0	0.0005
Pos. Control	Quinine		70.3	0.010	73.0	0.001

Table 4. Results of phytochemical screening

Plants	**Phytochemicals**				
	alkaloids	flavonoids	terpenes	tannins	Phenolic acids
A. chinensis	-	+	+	+	+
E. palustre	-	-	+	+	-
M. azedarach	-	+	+	+	-
P. angulata	-	+	+	+	+
S. icaja	+	±	±	-	+

Supplementary material

In vivo antimalarial activity (parasite growth inhibition) of extracts on mice infected by *Plasmodium berghei berghei*.



Blue = D8

Red = D9

Dose of extract: 300mg/kg

Quinine : 20mg/kg

Chloroquine: 25mg/kg