Global Journal of Medicinal Plants Research

ISSN: xxxx-xxxx Vol. 1 (1), pp. 038-044, December, 2013. © Global Science Research Journals

Full Length Research Paper

Antiplasmodial and repellent activity of indigenous plants used against malaria

M. Traoré-Coulibaly[1,2]*, L. Paré-Toé[1,2], H. Sorgho[1,2], C. Koog[1,5], A. Kazienga[1], K. R. Dabiré[1,2], L. C. Gouagna[1], P. Z. Dakuyo[3], J. B. Ouédraogo[1,2], I. P. Guissou[4] and T. R. Guiguemdé[2]

[1]Direction Régionale Institut de Recherche en Sciences de la santé (IRSS-DRO), Burkina Faso.
[2]Unité de Recherche Paludisme et Maladies Tropicales Négligées, Centre Muraz, Burkina Faso.
[3]Pharmacie de la Comoé, Burkina Faso.
[4]Institut de Recherche en Sciences de la santé (IRSS), Burkina Faso. [5]Institut de Recherche pour le Développement (IRD), Bobo-Dioulasso, Burkina Faso.
[6]Université Claude Bernard, Lyon I, Faculté de Pharmacie, Burkina Faso.

Accepted 21 October, 2013

Aqueous and organic extracts from *Desmodium velutinum* (Willd) DC (Fabaceae), *Combretum molle* R.Br ex G. Don, *Combretum sericeum* G. Don (Combretaceae), *Bidens engleri* O.E.Schulz (Asteraceae), *Cucumis metuliferus* E. Mey. ex Naudin, *Cassia podocarpa* Guill. et Perr. (Ceasalpiniaceae), and *Opilia celtidifolia* Guill. & Perr. (Opiliaceae) from Burkina Faso were prepared and tested *in vitro* against *Plasmodium falciparum* parasites. Chloroquine was used as a positive control. Essential oils extracted from *Hyptis suaveolens* and *Hyptis spicigera* used as repellent were screened against *Anopheles gambiae* under laboratory conditions using human subjects. Dichloromethane leaf extracts of *D. velutinum* and *C. sericeum* (IC_{50} = 9 µg/ml), and the dichloromethane root extract of *O. celtidifolia* (IC_{50} < 11 µg/ml) showed promising antiplasmodial activity. The ethanol leaf extracts of *C. podocarpa* (IC_{50} = 22 µg/ml), *D. velutinum* (IC_{50} = 36 µg/ml) and *C. molle* (IC_{50} = 25 µg/ml) showed moderate activity. Low activity was observed in the aqueous leaf extracts of *C. sericeum* (IC_{50} = 68 µg/ml), *D. velutinum* (IC_{50} > 100 µg/ml), *B. engleri* (IC_{50} = 101 µg/ml) and *C. metuliferus* (IC_{50} > 100 µg/ml). Repellency of the two essential oils was high during the first hour but decreased with the bioassay time. The relatively high repellency of *A. gambiae* mosquitoes estimated as 87.6 ± 0.2% (*H. spicigera*) and 81.7 ± 0.3% (*H. suaveolens*) confirms the traditional use of the two plants.

Key words: Malaria, antiplasmodial, *Plasmodium falciparum*, mosquito, repellent, *Anopheles gambiae*, plants.

INTRODUCTION

Malaria is a disease caused by a protozoan parasite of *Plasmodium* genus transmitted to humans by the bite of *Anopheles gambiae* female mosquitoes. Despite decades of worldwide control efforts, malaria is still causing up to 225 million episodes of illness and 800,000 deaths every year (World Health Organization (WHO), 2012) and sub-Saharan Africa has the highest burden of human suffering. In Burkina Faso, 17% of the population is reported to be infected with malaria each year and approximately 5000 die from the disease (Programme National de Lutte contre le Paludisme (PNLP), 2006). Empirical observations and ethnobotanical surveys have shown that tribal populations use decoctions or infusions of antimalarial herbs for treating malaria and repellent

*Corresponding author. E-mail: traore_maminata@yahoo.fr. Tel/Fax: +266-20974868

plants are used fresh or burned in rooms to get rid of mosquitoes. Repellents are substances applied to the skin, which prevent insects from biting and diethylbenzamine (DEET) which is the gold standard, is in use for more than 50 years (Hotzer, 2001). In the case of malaria, a safe and effective way of preventing *Anopheles* female bites is needed. In Burkina Faso,

Hyptis spicigera and *Hyptis suaveolens* were identified as repellent plants, while plant species such as *Desmodium velutinum* (Willd) DC, (Fabaceae), *Combretum molle* R.Br ex G. Don, *Combretum sericeum* G. Don (Combretaceae), *Bidens engleri* O.E.Schulz (Asteraceae), *Cucumis metuliferus* E. Mey. ex Naudin), *Cassia podocarpa* Guill. et Perr. (Ceasalpiniaceae) and *Opilia celtidifolia* Guill. & Perr. *(Opiliaceae)* are traditionally used against fever and malaria attacks (Guigma et al., 2012; Togola et al., 2005; Arbonnier, 2009).

In other African regions, *H. spicigera* leaves are used as a spray to keep and protect crops from various insect attacks and are placed in a layer below bundles of millet to keep away termites. Placing branches or whole plants inside houses is most common for *H. suaveolens* (Seyoum et al., 2002).

The increasing resistance of malaria parasites and mosquitoes to available drugs and insecticides, respectively has created a growing interest in herbal remedies in the hope of identifying new leads for antimalarial and repellent drug development. Plants are known to produce secondary metabolites that are found to be physiologically active and have been used for medicinal purposes for centuries. It is also known that the activity of aromatic repellent plants is due to essential oils present in the plant material and that are used as flavor in food products, odorants in flagrances, pharmaceuticals and as insecticides. Essential oils are natural volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes (Nerio et al., 2010). Mosquito repellent activity has been found in various plant extracts; among them *Azadirachta indica* A. Juss (neem tree), *Ocimum basilicum* L. (basil oil) and *Citronella* species (Kweka et al., 2008). This study investigates the antiplasmodial and repellent activity of crude extracts and essential oils derived from nine plants aiming to justify the traditional use of these plants in the control of malaria. No or very few pharmacological studies have been previously reported on the plants selected for the current study.

MATERIALS AND METHODS

Plants used to treat malaria

A series of plants claimed to cure malaria in traditional medicine were collected in different regions of Burkina Faso. Priority was given to the following species: *D. velutinum* (Willd) DC., (Fabaceae), *C. molle* R.Br ex G. Don, *C.sericeum* G. Don (Combretaceae), *B. engleri* O.E.Schulz (Asteraceae), *C. metuliferus* E. Mey. ex Naudin, *C. podocarpa Guill*. and Perr. (Ceasalpiniaceae) and *O. celtidifolia* Guill. & Perr. (Opiliaceae).

Plants used against mosquitoes

H. suaveolens (L) and *H. spicigera* (Lam), the plants most used by the community in the west part of the country, were chosen for the study.

Plant materials were collected and authenticated by an experienced botanist at Centre National la Recherche Scientifique et Technologique (CNRST). Voucher specimens were kept in the herbarium of CNRST under numbers presented in Table 1.

Extraction for malaria bioassay

Each ground plant material (100 g) was extracted by ethanol (1 L) for 24 h. Residue of some of the extracts was suspended in dichloromethane and extracted with water. All the extracts were concentrated in a Rotavapor system and stored for the test on malaria parasites.

Essential oil extraction

Essential oils were obtained from leaves of *H. spicigera* and *H. suaveolens* subjected to steam distillation for 1½ h using a Clevenger type apparatus located in Phytofla laboratories. The oils were collected, dried and stored in sealed vials at 4 to 6°C until use.

Antiplasmodial bioassay

The antiplasmodial activity was evaluated *in vitro* using *Plasmodium falciparum* chloroquine-sensitive strain (3D7) according to a method described in detail elsewhere (Ziegler et al., 2002) by using chloroquine as a positive control. Briefly, the parasites previously maintained in continuous culture were cultivated during 48 h in the presence of four different concentrations of each of the plant extract (100, 50, 25 and 12.5 and 6.25 µg/ml). The IC_{50} of each extract was then determined. The IC_{50} values of the extracts were then compared to the thresholds for *in vitro* antiplasmodial activity of plant extracts established by Rasoanaivo et al. (2004).

Repellent bioassay

Bioassays were performed to evaluate the repellent activity of essential oils from *H. spicigera* and *H. suaveolens* by recording the sensitivity of mosquitoes. Two to three days old strictly nulliparous female *Anopheles gambiae "Kisumu*" non-exposed before to human contact were used for the bioassay. This laboratory-susceptible reference colony is maintained in the insectarium of IRSS/Centre Muraz.

Evaluations were carried out on the arms of human volunteers inside cages (30 × 30 × 30 cm) containing mosquitoes according to the method described by Curtis et al. (1987) and WHO (1996). Both arms of human volunteers were carefully covered with thick paper except a 4 cm^2 skin portion exposed to mosquito bites. This skin portion was treated with each essential oil (4 µl/cm^2) and compared to the skin portion of the other arm treated by paraffin as control. Arms were simultaneously introduced into the cage containing 25 female *A. gambiae* mosquitoes and the number of bites was counted over 5 min. Assessment time-points were t_0 = 0.5, t_1 = 1.5, t_2 = 2.5 and t_3 = 3.5 post-application without renewing the essential oil treatment and each assay was repeated 5 times. All tests were conducted at the same room temperature (25 to 30°C) and humidity (60 to 80%).

T**able 1.** Effects of crude extracts and essential oils on *P. falciparum* and *A. gambiae* mosquito.

No.	Ref. (id.)	Species	Family	Plant part	Type of extract	IC_{50} (µg/ml)	Antiplasmo activity
1	8708	*Desmodium velutinum*	Fabaceae	Leaves	Water	>100	Inactive
					Ethanol	36	Weak
					Dichloromethane	9	Good to mod
2	8712	*Opilia celtidifolia*	Opiliaceae	Roots	Water	>100	Inactive
					Dichloromethane	<11	Good to mod
3	8713	*Combretum sericeum*	Combretaceae	Leaves	Water	68	Very weak
					Ethanol	>100	Inactive
					Dichloromethane	9	Good to mod
4	8705	*Cassia podocarpa*	Ceasalpiniaceae	Leaves	Ethanol	22	Weak
5	3348	*Cucumis metuliferus*	Curcurbitaceae	Leaves	Ethanol	>100	Inactive
6	8707	*Bidens engleri*	(Asteraceae)	Leaves	Ethanol	101	Inactive
7	5859	*Combretum molle*	Combretaceae	Leaves	Ethanol	25	Weak
8	2340	*Hyptis spicigera*	Lamiaceae	Leaves	Oil	-	-
9	2341	*Hyptis suaveolens*	Lamiaceae	Leaves	Oil	-	-
-	-	Chloroquine	-	-	-	3-14 ng/ml	-

Ref. = Reference, Id = identification.

Statistical analysis

Antiplasmodial activity was assessed through the IC_{50} determined from dose-response curves. The repellency index (R) of the essential oils against *A. gambiae* mosquito species was estimated by the equation as follows:

$$R (\%) = [(C–T)/C] \times 100$$

where C and T are the number of mosquitoes collected on the control and the treatment arms respectively.

Statistical analysis was performed using Stata version 10. Comparisons of repellence index within and between plant species were carried out using the Student's t-test. All differences were considered significant at $P<0.05$.

Ethical approval

The repellent protocol was reviewed and approval by the Institutional Ethics Committee of Centre Muraz, Bobo-Dioulasso.

RESULTS AND DISCUSSION

Antiplasmodial activity

The aqueous as well as organic extracts tested against malaria parasites in the present study showed a variable level of inhibitory activity according to the plant extracts as shown in Table

1. The dichlor
velutinum, C. se
most active. P
significant antip
extract of C*. mo*
plasmodial activ
tannins isolated
et al., 2001), wh
activity of the
moderate activit
extract, IC_{50}=5.
IC_{50}=7.9 µg/ml
Gansané et al. (

Extracts from

Table 2. Mean number of *Anopheles gambiae* bites per plant at various post exposure time.

Time post application of repellent (h)	Mean number of mosquito bites					
	Hyptis spicigera			*Hyptis suaveolens*		
	Test arm	Control arm	Number of repeat	Test arm	Control arm	Number of repeat
0.5	0.8 ± 1.09	5.6 ± 2.07	5	0.8 ± 1.09	4.8 ± 3.11	5
1.5	2.2 ± 0.47	7.4 ± 3.57	5	1.6 ± 1.51	6.6 ± 3.97	5
2.5	6.6 ± 1.67	14.6 ± 3.64	5	4.4 ± 2.70	12.2 ± 2.77	5
3.5	8.5 ± 2.64	14.75 ± 3.40	4	6.6 ± 3.97	11.2 ± 2.48	5

strains *of P. falciparum* were not found to inbihit the parasites very actively (Koudouvo et al., 2011). The use of wild strains may explain the high IC_{50} obtained.

A preliminary phytochemical screening conducted by Sini (2008) revealed the presence of tannins, flavonoids, glycosides, anthraquinones and alkaloids in the aqueous root extract of *C. sericeum*. The antiparasitic activity of alkaloids and anthraquinones has been described several times (Addae-Kyereme et al., 2001). No phytochemical investigation was conducted with *D. velutinum,* but compounds such as triterpenoids, saponins, polyphenols, flavonoids, anthocyanins, alkaloids and tannins have been identified from the genus *Desmodium* (Muanda et al., 2011). Phytochemical studies on *O. celtidifolia* revealed the presence of six triterpenoid saponins from the leaves and stems of the plant (Crespin et al., 1993); polysaccharides from the leaves used for wound healing in traditional medicine in Mali (West Africa) were found to possess a potent activity in the complement system (Togola et al., 2006). Latest studies have shown anti-helminthic, uterine stimulant, hypotensive and cardiac depressant activities of a methanol extract of the stem bark containing mainly saponins (Shibata et al., 1977). The presence of such compounds in the active plant extracts may explain their potency. Extracts with promising antiplasmodial activity (IC_{50}≤10 µg/ml), that is, dichloromethane leaf extracts of *D. velutinum (*IC_{50}=9 µg/ml), *C. sericeum* (9 µg/ml) and dichloromethane root extract of *O. celtidifolia* (<11 µg/ml) will be selected for further bioassay guided fractionation for the identification of potent ingredients. Extracts with moderate activity will be subjected to an *in vivo* study in mice to confirm the antimalarial activity.

Mosquito repellency of the crude essential oils of *H. spicigera* and *H. suaveolens*

Natural plant extracts have been used for centuries by human populations to prevent arthropod bites. Many preparations from naturally occurring sources are repellent to different insect species; some of these act as insecticides while others are only repellent. Natural repellent oils were found to be unstable and did not provide good repellency, since they can evaporate completely. Synthetic repellents were also found to be more effective than the natural ones. Usually, synthetic substances showed 100% repellency for the first 2 h, where the known natural repellent products were most effective for the first 30 to 60 min and reapplication is then necessary to maintain the efficacy over several hours (Patel et al., 2012). Neem oil (*Azadirachta indica*) repels or kills mosquitoes, their larvae and a plethora of other insects including those in agriculture, while Citronella oil repels mosquitoes (Patel et al., 2012). Neem oil was found to be mosquito repellent for up to 12 h. Cinnamon leaf oil, Clove oil and Eucalyptus oil containing cineol kill mosquito larvae and adults (Patel et al., 2012; Koul et al., 2008).

H. spicigera and *H. suaveolens* are plants burned in rooms to repel mosquitoes. To test this claim the essential oils of the two plants were selected and tested for mosquito repellency on human volunteer using the method described earlier. No skin irritation, rashes were observed on the arms of the treated volunteers during all the duration of the bioassay (4 h). The mean number of *A. gambiae* bites per plant is presented in Table 2. The results revealed that the essential oils derived from *H. spicigera* and *H. suaveolens* were effective against *A. gambiae*. Indeed, analysis has shown a significant difference between the control and the tested arm during the bioassay (Table 2). High percentage repellency (*H. spicigera* (87.6 ± 0.2%) and *H. suavolens* (81.7± 0.3%) was obtained with the two plant essential oils at the beginning of the assay, but decreased with the bioassay time (Figure 1). Each oil was effective for 2.5 h with more than 50% repellency. Comparison of the plants did not reveal any significant difference between the percentage repellency of the essential oils derived from the two plants, which seem to have the same repellency ($P > 0.05$). Very few studies have demonstrated the mosquito repellency effect of the two plants. *H. suaveolens* thermally expelled in experimental huts within a screenwalled greenhouse provide a low repellent effect against *A. gambiae* (Seyoum et al., 2002). Our results were comparable to those obtained by other authors (Abagli et al., 2012; Kweka et al., 2008) obtained with essential oils from *Ocimum suave* and *Ocimum kilimandscharicum* and *H. suavolens* 71.2 to 88.9% biting protection against three species of mosquitoes, *A. gambiae* ss, *Anopheles arabiensis* and *Culex quinquefasciatus*. Conti et al. (2012) obtained a protection

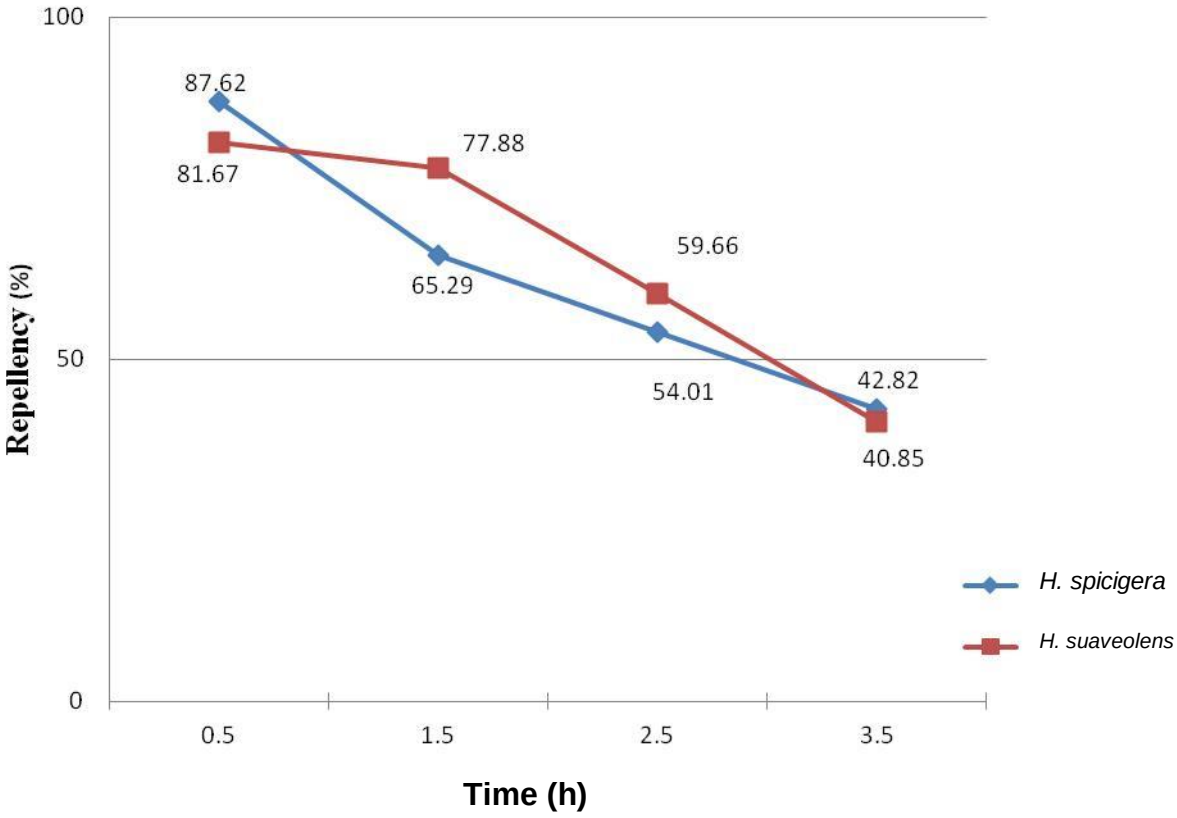

Figure 1. Relative efficacy of the essential oils of *H. spicigera* and *H. suaveolens* against *Anopheles gambiae* mosquito growth in laboratory.

time ranging from 16 to 135 min with the essential oil of *H. suaveolens*. The insecticide activity of *H. spicigera* and *H. suaveolens* on other insect species has been previously demonstrated. Crude essential oils of *H. spicigera* were found to cause 100% mortality of early-stage larvae and young adults of *Tribolium castaneum (*Kouninkie et al., 2007). Essential oil of *H. spicigera* applied as fumigant was found to induce between 95 and 96% mortality on the Coleopteran insect *Sitophilus zeamais* Motsch*.* Against *Sitophilus oryzae L*., the plant essential oil exhibited a repellency activity with a repellent percentage (RP) of 62.5% (Ngassouma et al., 2007). Some factors such as the mosquito species, the volunteer age, sex temperature, humidity, wind and biochemical attractiveness of the tested mosquito were found to play a role in the effectiveness of repellent compound or extract; this may explain the variation observed between the tests during the 5 days. The chemical content of *H. spicigera and H. suaveolens* essential oil has been established over the years from previous phytochemical studies; the two plant species were found to contain numerous volatiles known to have pesticide and/or insect repellent properties. The essential oil of *Hyptis suaveolens* was also found to possess antioxidant and antimicrobial activities. The main active compounds identified from the species were sabinene, α-terpinolene and 1,8-cineole (Nantitanon et al., 2007). *Hyptis suaveolens* collected in the Brazilian Cerrado region revealed the presence of sabinene, limonene, biclyclogermacrene, beta-phellandrene and 1,8-cineole (Azevedo et al., 2001). Leaves of *H. suaveolens* collected in Guinea-Bissau were found to contain mainly β-caryophyllene, bergamotene, and terpinolene (Jaenson et al., 2006). Seven labdane diterpenes with insecticidal properties were isolated from the aerial parts of *H. spicigera* by Fragoso-Serran et al. (1999). More than 48 compounds have been identified from *H. spicigera*; among them γ-terpinene, α and β-pinene, p-cymene, eucalyptol or 1,8- cineole and β-caryophyllene. Investigation of the essential oils by GC-MS of *H. spicigera* from Cameroon revealed the presence of two main components: 1,8-cineol (24.0%) and (E)-caryophyllene (22.2%). Other active compounds identified in the essential oil were α-pinene (9.1%), β-pinene (5.7%), α-terpineol (8.3%) and linalool (8.4%) (Ngassouma et al., 2007). Belanger et al. (1994) identified the presence of α-pinene (5.1%), β-pinene (3.2%) and β-caryophyllene (65.7%) in *H. spicigera* from Burkina Faso. Bioactivity-guided fractionation of the petroleum ether extract of the leaves of *H. suaveolens* led to the isolation of an abietane-type diterpenoid endoperoxide, 13 alpha-epi-dioxiabiet-8(14)-en-18-ol, displaying high antiplasmodial

activity (IC_{50}=0.1 µg/ml) (Chukwujekwu et al., 2005). The same repellency displayed by the two plant essential oils may be due to the relative similarity of their chemical components.

Conclusion

Our results indicated that the dichloromethane extracts of *D. velutinum*, *C. sericeum*, and *O.celtidifolia* were more effective in terms of antiplasmodial action against *P. falciparum* compared to the ethanol and water extracts of the same or other plants (*C. molle*, *C. metuliferus*, *B. engleri* and *C. podocarpa)*. 87.6 and 81.7% repellency against *A. gambiae* adult mosquitoes reared in the laboratory were obtained respectively with *H. suaveolens* and *H. spicigera* essential oils. At least 50% of the repellency is maintained for 2½ h. These values may be compared to those of some synthetic insect repellents tested in the same conditions. These plant oils may represent an alternative in formulating potent and affordable products in the control of malaria mosquitoes.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge laboratory Phytofla for permanent support, all involved research centers for their collaboration. Many thanks to Soren Brogger Christensen, Dorte Brix (Pharmaceutical University of Copenhagen) and Belem Mamounata (CNRST –Burkina) for technical support.

REFERENCES

Abagli AZ, Alavo TB, Avlessi F, Moudachirou M (2012). Potential of the bush mint, *Hyptis suaveolens* essential oil for personal protection against mosquito biting. J. Am. Mosq. Control Assoc. 28(1):15-19.

Addae-Kyereme J, Croft SL, Kendric HK, Colin W (2001). Antiplasmodial activities of some Ghanaian plants traditionally used for fever/malaria treatment and of some alkaloids isolated from *Pleiocarpa mutica; in vivo* antimalarial activity *of pleiocarpine.*, J. Ethnopharmacol. 76(1):99–103

Arbonnier M (2009). Arbres, arbustes et lianes des zones sèches d'Afrique de l'ouest. Nature. pp. 519-544.

Asres K, Bucar F, Knauder E, Yardley V, Kendrick H, Croft SL (2001). *In vitro* antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. Phytother Res. 15(7):613-617.

Azevedo NR, Campos IF, Ferreira HD, Portes TA, Santos SC, Seraphin JC, Paula JR, Ferri PH (2001). Chemical variability in the essential oil of *Hyptis suaveolens*. Phytochemistry 57(5):733-736.

Belanger A, Dextraze L, Nacro M, Samaté AD, Collin G, garnaux FX, Gagnon H (1994). Compositions chimiques d'huiles essentielles de plantes aromatiques du Burkina Faso. Actes des 13iemes Journées internationales des Huiles essentielles. Digue-les- Bains, France pp. 300-305.

Chukwujekwu JC, Smith P, Coombes PH, Mulholland DA, van Staden J (2005). Antiplasmodial diterpenoid from the leaves of *Hyptis suaveolens*. Ethnopharmacol. 14,102(2):295-297.

Conti B, Benelli G, Flamini G, Cioni PL, Profeti R, Ceccarini L, Macchia M, Canale A (2012). Larvicidal and repellent activity of Hyptis suaveolens (Lamiaceae) essential oil against the mosquito Aedes albopictus Skuse (Diptera: Culicidae). Parasitol. Res.110(5):2013-21.

Crespin F, Olliver E, Lavaud C, Babadjamian A, Faure R, Debrauwer L, Balansard G, Boudon G (1993). Triterpenoid saponins from Opilia celtidifolia. Phytochemistry 33(3):657-661.

Curtis CF, Lines JD, Ijumba J, Callanghan A, Hill N, Karimzad MA (1987). The relative efficacy of repellents against mosquito vectors of disease. Met. Vet. Entomol. 1:109-119.

Fragoso-Serrano M, Gonzalez-Chimeo E, Pereda-Miranda R (1999). Novel labdane diterpenes from the insecticidal plant *Hyptis spicigera* Nat. Prod. 62(1):45-50.

Gansané A, Sanon S, Ouattara LP, Traoré A, Hutter S, Ollivier E, Azas N, Traore AS, Guissou I, Sirima SB, Nebié I (2010). Antiplasmodial activity and toxicity of crude extracts from alternatives parts of plants widely used for the treatment of malaria in Burkina Faso: contribution for their preservation. Parasitol. Res. 106(2):335-40.

Guigma Y, Zerbo P, Millogo-Rasolodimby J (2012). Utilisation des espèces spontanées dans trois villages contigus du Sud du Burkina Faso. Tropicultura 30(4):230-235.

Holzer RB (2001). Protection against biting mosquitoes. Ther. Umsch. 58(6):341-346.

Jaenson TG, Pålsson K, Borg-Karlson AK (2006). Evaluation of extracts and oils of mosquito (Diptera: Culicidae) repellent plants from Sweden and Guinea-Bissau. J. Med. Entomol. 43(1):113-119.

Koudouvo K, Karou SD, Ilboudo DP, Kokou K, Essien K, Aklikokou K, de Souza C, Simpore J, Gbéassor M (2011). *In vitro* antiplasmodial activity of crude extracts from Togolese medicinal plants. Asian Pac. J. Trop. Med. 4(2):129-32.

Koul O, Walia S, Dhaliwal GS (2008). Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4(1):63–84.

Kouninkie H, Ngamo LST, Hance T, Ngassoum, MB (2007). Potential use of essential oils from local cameroonian plants from the control of red flour weevil *Tribolium Castaneum* Sp (Herbst.) (Coleoptera : Tenebrionidae). Afr. J. Food Agric. Nutr. Dev. 7:5.

Kweka EJ, Mosha F, Lowassa A, Mahande AM, Kitau J, Matowo J, Mahande MJ, Massenga CP, Tenu F, Feston E, Lyatuu EE, Mboya MA, Mndeme R, Chuwa G, Temu EA (2008). Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania. Malar. J. 7:152.

Muanda FN, Bouayed J, Djilani A, Yao C, Soulimani R, Dicko A (2011). Chemical Composition and, Cellular Evaluation of the Antioxidant Activity of *Desmodium adscendens* Leaves. Evid. Based Complement. Alterna.t Med. 2011:620862.

Nantitanon W, Chowwanapoonpohn S, Okonogi S (2007). Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential Oil. Scientia Pharmaceutica (Sci. Pharm.) 75:35-46.

Nerio LS, Olivero-Verbel J, Stashenko E (2010). Repellent activity of essential oils: a review. Bioresour Technol. 101(1):372-378.

Ngassouma MB, Ngamo TLS, Ngatanko I, Tapondjoud LA., Lognaye G, Malaisse F, Hance T (2007). Chemical Composition, Insecticidal Effect and Repellent Activity of Essential Oils of Three Aromatic Plants, Alone and in Combination, towards Sitophilus oryzae L. (Coleoptera: Curculionidae). Natural Product Communications 2(12):1229-1232.

Patel EK, Gupta A, Oswal RJ (2012). A review on mosquito repellent methods. Int. J. Pharmaceut. Chem. Biol. Sci. 2(3):310-317.

Programme National de Lutte contre le Paludisme (PNLP) (2006). Plan stratégique 2006-2010 de lutte contre le paludisme au Burkina Faso p. 53.

Rasoanaivo P, Deharo E, Ratsimamanga-Urverg S, Frappier F (2004). Guidelines for the non-clinical evaluation of the efficacy of traditional antimalarials. In: Willcox M.L, Bodeker G and Rasoanaivo P (Eds). Traditional Medicinal Plants and Malaria. Boca Raton: CRC Press pp. 256-268.

Seyoum A, Pålsson K, Kung'a S, Kabiru EW, Lwande W, Killeen GF, Hassanali A, Knols BG (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. Trans. R. Soc. Trop. Med. Hyg. 96(3):225-231.

Shibata IM, El-Gendi AY, Abdel El-Malik MM (1977). Pharmacological studies on saponin fraction of *Opilia celtidifolia.* Planta Med. 31:60-67.

Sini JM, Umar IA, Anigo KM, Stantcheva I, Bage EN, Mohammed R

(2008). Antidiarrhoeal activity of aqueous extract of Combretum *sericeum* roots in rats. Afr. J. Biotechnol. 7 (17):3134-3137.

Togola A, Diallo D, Dembélé S, Barsett H, Paulsen BS (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. J. Ethnobiol. Ethnomed. 1:7.

Togola A, Diallo D, Michaelsen TE, Paulsen BS (2006). Structure elucidation of bioactive pectins from *Opilia celtidifolia* (Guill. & Perr.) Endl. Ex Walp. (Opiliaceae). Planta Med. p. 72.

WHO (1996). Report of the WHO informal consultation on the evaluation and testing of insecticides.Geneva, Switzerland: World Health Organization, Control of Tropical Diseases Division. CTD/WHOPES/IC/96.1.

WHO (2012). World Malaria Report, 272PZiegler HL, Stærk D, Christensen J, Hviid L, Hägerstrand H, Jaroszewski JW (2002). *In vitro P. falciparum* drug sensitivity assay: inhibition of parasite growth by incorporation of stomatocytogenic amphiphiles into erythrocyte membrane. Antimicrob. Agents Chemother. 46:1441-1446.