ISSN 0974 – 5211

Journal of Natural Products
Volume 4 (2011)
www.JournalofNaturalProducts.Com

Research Paper

In vitro and in vivo antiplasmodial activities of extracts of *Cymbopogon citratus* Staph and *Vernonia amygdalina* Delile leaves

Paula Melariri[1*], William Campbell[1], Paschal Etusim[2], Peter Smith[1]
[1] Division of Pharmacology, Department of Medicine, University of Cape Town Medical School K45, Old Main building Groote Schuur Hospital, Observatory 7925, South Africa
[2]Abia State University Uturu, PMB 2000 Uturu, Abia State, Nigeria
* Corresponding Author
(Received 08 June 2011; Revised 09 June-04 July 2011; Accepted 05 July 2011)

ABSTRACT

Present study investigated the *in vivo* antiplasmodial activity of *C. citratus* and *V. amygdalina* leaves extracts when used in combination in mice. Greatest *in vitro* antimalarial activity occurred in the dichloromethane extracts which were investigated for *in vivo* antimalarial activity in mice at doses of 400mg/kg and 600mg/kg. A significant dose dependent activity was observed. 600mg/kg of each extract used in combination and administered orally successfully suppressed parasitemia in mice for greater than 30 days compared to controls which died within ten days. No recrudescence was observed and mice maintained their weight. These results show that mice infected with *P. berghei* can be cured by a combination of extracts of the two Nigerian plants *C. citratus* and *V. amygdalina* at a dose of 600 mg/kg using a 4-day suppressive treatment.

Keywords: *In vivo;* Antiplasmodial; *Cymbopogon citratus*; *Vernonia amygdalina*; Leaves.

INTRODUCTION

Cymbopogon citratus (Family- *Poaceae*) used in tropical and subtropical countries and in producing a pleasant aroma in their herbal teas (Cheel, et al., 2005). It's also used in the treatment of fever, jaundice, hypertension, as analgesic, in soap making and as an insect repellant (Onabanjo, et al., 1993), ameliorates nervous and gastrointestinal abnormalities (Melo, et al., 2001). Their essential oil have antibacterial and antifungal, activity (Suhr and Nielsen, 2003; Wilkinson and Cavanagh, 2005). Its sedative and anticonvulsant properties as well as its use as an anxiolytic agent has been documented (Blanco, et al., 2009).

Vernonia amygdalina, (Family- *Asteraceae*) locally known as "omubirizi", and is traditionally used for analgesia and in the treatment of malaria infections (Anoka, et al., 2008). It is used as anthelmintic, antiprotozoal and antibacterial agent (Huffman et al., 1996a). The antitumour and antimicrobial properties of *V. amygdalina* have been traced to its bioactive principles (Koshimizu, et al., 1994). It has hypoglycemic, antineoplastic and antibacterial properties (Izevbigie, et al., 2004; Iwalewa, et al., 2003). *V. amygdalina* has shown antiplasmodial properties, which has

been attributed to the sequesterpene lactones isolated from the leaves (Masaba, 2000; Abosi and Raseroka, 2003; Tona et al., 2004). This species has been shown to enhance the *in vivo* activity of chloroquine (Iwalokun, 2008). *Cymbopogon citratus* Staph *and Vernonia amygdalina* Delile investigated in this study are used in the traditional treatment of malaria in Nigeria. However studies on the *in vivo* antiplasmodial activity of a combination of dichloromethane extracts from the leaves of *Cymbopogon citratus* and *Vernonia amygdalina* are nonexistent in the literature.

Combination therapies are a vital strategy to prevent or delay resistance of parasites and have been approved for other multidrug resistant infections, such as HIV and tuberculosis. Although the combination of compounds for the treatment of malaria has been recommended (White, 1998; WHO, 2001), the current research is aimed at evaluating the *in vivo* antiplasmodial activities of the dichloromethane extracts *C. citratus* and *V. amygdalina* when used in combination.

MATERIALS AND METHODS

***Plant materials*:** In Nigeria where these plants were collected from, there are two major seasonal variations, the rainy season and the dry season. The plants leaves were collected during the long rainy season (March to July). Plants (voucher specimens number: PM/ABSU/06-72 and PM/ABSU/06-82 respectively) were identified by the taxonomist in Plant Science and Biotechnology Dept., Abia state University Uturu.

***Extraction*:** The fresh leaves of plants were dried for 3 weeks and powdered using a plant blender (Waring, Connecticut, USA). The dried and powdered leaves of *Cymbopogon citratus* and *Vernonia amygdalina* were sequentially and exhaustively extracted using solvents of different polarities i. e. petroleum ether, dichloromethane, ethyl acetate, methanol and water. For a proper mixing plant material and the solvent were continuously shaken on a horizontal orbit shaker (Labcon, California, USA). The resultant mixture was filtered and the filtrate concentrated under pressure in a Büchi Rotavapor R-205 (Büchi Labortechnik AG Switzerland), at 24ºC.

***Animals and diet*:** This study was approved by the Animal Research Ethics Committee of the University of Cape Town, South Africa in 2007. All animal procedures were carried out in accordance with the suggested ethical guidelines for care of laboratory animals by the Animal Care and Use Committee of the University of Cape Town, as adapted from the recommendations of the Medical Research Council of South Africa [MRC 2004]. The test animals were wild strains of C57 BL6 mice. Mice were obtained from the animal unit of the University of Cape Town (South Africa) at the ages between 7-10 weeks old. They were housed in standard cages in groups of five and placed on a pelleted custom diet. They were maintained under conventional conditions with controlled temperature (22±4ºC) and illumination (12h; 6:00 am to 6:00 pm) and had free access to standard diet and water *ad libitum*.

***Parasite strain*:** The parasites used for this experiment were of the cryopreserved *P. berghei* (ANKA) strain. This parasite strain was donated by the Swiss Tropical Institute, Basel, Switzerland. Parasite stock was preserved in liquid nitrogen at -80ºC in the Division of Clinical Pharmacology, University of Cape Town. Parasite stock was sustained by serial passage of blood from infected mice to uninfected mice. Parasitemia was monitored regularly. At a desired parasitemia, the mice were bled and euthanized. Blood samples collected were frozen in cryotubes and stored in liquid nitrogen at -80ºC.

***Acute oral toxicity study of crude extracts*:** Acute oral toxicity testing of extracts in mice was investigated at a dose of 1600mg/kg for 4 days. The extracts dissolved

completely in 200µl ethanol, 20µl Tween 80 and 780µl phosphate buffered saline (PBS). The dosage was administered orally. The test animals were monitored for 4 weeks to ascertain if there were any adverse events. We used the same formulation for the *in vivo* antiplasmodial study of the extracts in this work.

***In vitro antiplasmodial assay*:** was carried out as described by Makler et al., 1993.

In vivo antiplasmodial assays and extract administration: We studied the antiplasmodial activities of the dichloromethane extracts *C. citratus* and *V. amygdalina* when used singly and in combination at different doses. The dosage of the extracts was determined from preliminary studies in our laboratory. Having investigated the toxicity of these plants in mice model at higher doses >1500mg/kg, the extracts from these plants were administered orally at a dose of 800mg/kg for single extract treatment and in combinations, at two different doses (600mg/kg and 400 mg/kg) against chloroquine sensitive *P.berghei* (ANKA strain). Extracts completely dissolved in SMEDDS formulation (200µl ethanol, 20µl Tween 80, and 780µl phosphate buffered saline). Solvent control test for the formulation (200µl ethanol, 20µl Tween 80, and 780µl phosphate buffered saline) used in dissolving the extracts showed no antiplasmodial activity. The *in vivo* testing of crude extracts against *Plasmodium berghei* was carried out using the 4-day suppressive test described by Peters et al. (1993). Malaria infection was established in mice by the intraperitoneal (i.p) inoculation of 200 µl of $1x10^6$ parasitized cells/ml on the first day (D_0) of the experiment. Each mouse in the test group received 200µl of extract formulation 24 hours post infection. The dose was maintained for 4 consecutive days. Extracts were administered orally. The test animals were uniformly treated with different doses of extracts in combination. To ascertain the parasitemia, on day 3 of experiment, thin blood smear were made and stained with 10% Giemsa in Phosphate buffer, pH 7.2 for 20 minutes. The slide was examined under microscope at 100_X. The Percentage parasitemia was determined by counting the parasitized red blood cells on at least 1,000 red blood cells in random fields of the Giemsa stained slide. Each experiment had a positive control group and a negative control group. The positive control group received 200 µl of CQ (reference drug) at a dose of 10 mg/kg in Millipore water while the negative control group received 200 µl Millipore water only. The percentage growth inhibition was determined according to Tona et al., (2001) as follows;

$$\% \text{ growth inhibition} = \frac{\text{Parasitemia of negative control} - \text{Parasitemia of test sample}}{\text{Parasitemia of negative control}} \times 100$$

***Statistical analysis and data evaluation*:** Parasitemia of all groups was monitored, and growth inhibition calculated, as shown earlier. The standard deviation values of parasitemia and weight were determined using the Microsoft Excel® 2002. The percentage parasitemia relative to the number of days post infection was evaluated using the Graph Pad Prism 4 version.

RESULTS

***In vitro activity*:** When tested against the chloroquine sensitive strain of *P. falciparum*, the petroleum ether, ethyl acetate, and methanol extracts of *C. citratus* showed IC_{50s} of 9.1µg/ml, 12.1µg/ml, 15.9µg/ml respectively while the petroleum ether, ethyl acetate, and methanol extracts *V. amygdalina* recorded IC_{50}s of 14.1 µg/ml, 10.7µg/ml and >50µg/ml respectively. Water extracts of both plants showed IC_{50} > 50µg/ml. The dichloromethane extracts of the two plants recorded the greatest antiplasmodial activity *in vitro* and was further investigated *in vivo* in mice.

***Acute oral toxicity of crude extracts*:** These plants in combination showed no toxicity to mice. All animals gained body weight progressively. However, the evaluation approach used here is likely to demonstrate acute toxicity and not chronic effects of the extracts on vital organs in the body such as the brain, liver, kidney, or even the central nervous system. Assessment of the functional state of these organs, size and shape, as well as full blood count, may offer a better and more reliable assessment of *in vivo* toxicity.

***In vivo antimalarial activities*:** In the present study, there was a marked growth inhibition of parasites with values of 87.2% and 95.8% by the dichloromethane extracts of *C. citratus* and *V. amygdalina*, respectively at a dose of 800 mg/kg. The growth of the parasites treated with *C. citratus* and *V. amygdalina* was highly restricted, and most of them appeared as "dots" during the course of treatment. The two groups of test animals treated with either of these two extracts outlived all others, including the group which received chloroquine. Recrudescence was delayed in the groups treated with *C. citratus* or *V. amygdalina* when compared to other groups.

Having investigated the toxicity of these plants in mice at higher doses >1500mg/kg, *C. citratus* (DCM) + *V. amygdalina* (DCM) extracts were combined at two different doses (400 mg/kg and 600mg/kg). The group which received 400mg/kg of each extract showed parasite growth inhibition of 85% on day 3 of treatment, while the group treated with 600mg/kg inhibited the growth of the parasite by 95% (Table 1). The combinations which were tested at a dose of 600mg/kg of each extract showed good *in vivo* antiplasmodial activity. The mice treated with this combination showed no parasites after the 4 days of treatment. On day 1 post treatment there was a 100% growth inhibition of parasites in the group treated with *C. citratus* and *V. amygdalina* (600mg/kg each), while the group that received CQ (10mg/kg) and mice treated with 400mg/kg of each extract recorded 70% and 87% growth inhibition respectively (Table 1). A repeat experiment using the same dosage and experimental conditions yielded the same results. The repeat experiment recorded 100% parasite inhibition day 1 post treatment as was observed in the first experiment. Mice were monitored for 37 days. No recrudescence was seen in any of the mice in that group. The mice treated with a combination of *C. citratus* (DCM) and *V. amydalina* (DCM) at a dose of 600 mg/kg increased in weight by about 13% compared to their original weight before infection. The rest of the other groups died due to increased parasitemia, including the CQ group which recorded a high recrudescence post treatment. The weight details are shown in table 2.

DISCUSSION

A previous study which investigated the activities of *V. amygdalina* and *A. indica* in combination demonstrated that *V. amygdalina* alone is effective in reducing the blood glucose while *A. indica* effective in protecting the liver against damage in diabetic states, however they stated that a combination of the two extracts proved more potent in management of diabetes (Patrick, et al., 2008). In present study, mice treated with a combination of *C. citratus* and *V. amygdalina* extracts at a dose of 600 mg/kg each showed no parasitemia after treatment using the 4-day suppressive test. This was not the case when the extracts were administered singly. This observation compares well with the advantages of polyherbal therapies over monotherapy (Tiwari and Rao, 2002). However, those treated with the same combination at a dose of 400 mg/kg each showed high parasitemia post treatment. This suggests that the activity of the extracts is dose dependent. The dose dependent antimalarial activity of *C. citratus* essential oil

was shown in a previous study (Tchoumbougnang, et al., 2005). Similarly, antimalarial activities of acetone-water and aqueous extracts of *Vernonia amygdalina* green leaves showed a dose dependent activity against a chloroquine-sensitive isolate of *Plasmodium falciparum* (Masaba, 2000). These researchers observed that antimalarial activity was detected in both extracts at 10, 50, 100 and 200µg/ml in a dose dependent manner. They demonstrated that 25.5 and 76.7µg/ml of the acetone-water and aqueous extracts, respectively, would inhibit parasite growth by 50%. The activity of the aqueous extract in the previous study compares well to the activities of water extracts of the two plants investigated in the present study which showed IC_{50}>50µg/ml. The Stronger antiplasmodial activities displayed by the dichloromethane extracts suggests that the active components are highly lipophilic and may only be extracted with the non-polar solvents. Poor activity of the water extract from plants has been shown in several studies (Francois, et al., 1996; Clarkson, et al., 2004).

The plants investigated in this study are traditionally prepared as aqueous decoctions which involve boiling the plant and drinking the extract. More so, the traditional healers make use of these plants in their fresh state. The hot water treatment of these extracts could extract lower concentrations of active lipophilic components, but may not be available at therapeutic doses. It could be possible that preparing these plants in their fresh state, following the traditional recipe which involves boiling for several hours may have increased their activities. However, there is a possibility of denaturing some active components in the crude extracts since their heat tolerance has not been established.

V. amygdalina has also been shown to enhance the *in vivo* activity of chloroquine (Iwalokun, 2008). In a 4-day suppressive test, the ethanol leaf extracts and root bark of *V. amygdalina* suppressed parasites by 67% and 53.5%, respectively (Abosi and Raseroka, 2003), at a dose of 500mg/kg and 250mg/kg.

In present study there was a notable increase in weight of mice treated with a combination of *C. citratus* (DCM) and *V. amydalina* (DCM) at a dose of 600mg/kg when compared to the other group which received 400mg/kg. This may suggest that *V. amygdalina* contains some active biomolecules necessary for growth. Phytochemical analysis of *V. amygdalina* has shown the presence of several bioactive principles, which include bitter sesquiterpene lactones, vernodalin, vernolide and hydroxyvernolide, as well as steroid-related constituent's vernonioside BI and vernoniod BI (Koshimizu, et al., 1994). The antiplasmodial activity of *V. amygdalina* has been attributed to the sequesterpene lactones isolated from the leaves (Masaba, 2000; Abosi and Raseroka, 2003; Tona, et al., 2004). Previous study reported that sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodalin and hydroxyvernodalin isolated from *Vernonia amygdalina* leaves showed an antiplasmodial activity (IC50<4µg/ml) against *Plasmodium falciparum* strains (Phillipson et al., 1993). Similarly other extracts from *Vernonia* species such as the *n*-hexane from leaves of *Vernonia brasiliana* (L) Druce showed an in vitro antiplasmodial activity which was attributed to lupeol (Alves, et al., 1997). Numerous researchers have also recorded the antimlarial activity of the sesquiterpenes (Pedersen, et al., 2009; Topcu, et al., 2003).

The lipid composition of *V. amygdalina* was shown to contain 12 fatty acids and amounts to 74.1% of the lipid content (Erasto, et al., 2007a; b). Fatty acids were reported to cause degeneration in the intra erythrocytic stages of *P. falciparum in vitro*

(Kumaratilake, et al., 1992). The fatty acids could activate the neutrophils and their effector cells thereby enhancing their antimalarial properties.

The *in vivo* activity of *C. citratus* has been reported to show IC_{50}s from 6-9.5µg/ml with a 20µg/ml chloroform/ ethanol extract (Tchoumbougnang, et al., 2005). Five *C*-glycosylflavonoids from; orientin, isoorientin, isoscoparin, swertiajaponin and isoorientin 2˝-*O*-rhamnoside have been isolated from *C. citratus* and have shown potent antioxidant activity by significantly inhibiting lipid peroxidation in erythrocytic membranes (Orrego, et al., 2009). This could suggest that the recorded activity observed in the present study, using a combination of *C. citratus* and *V. amygdalina* at a dose of 600mg/kg each, may be traceable to the activity of the various compounds in the mixture which may have acted synergistically and such interactions between compounds could give rise to entirely new compounds with different physical and chemical properties.

CONCLUSION

To the best of our knowledge this is the first study which has shown that mice infected with *P. berghei* could be cured by a combination of the leaves extracts of two Nigerian plants *C. citratus* and *V. amygdalina* at a dose of 600mg/kg using a 4-day suppressive treatment. The number of existing antimalarials is still inadequate especially in the face of parasite resistance. The results of this study could help encourage more identification and validation of natural products which has shown antiparasitic properties thus facilitating the development of a new generation of antimalarials. The murine model of PbA in (BALB/C x 57BL/6) has shown several features in common with human cerebral malaria. Results from the use of these models may not be directly extrapolated to humans. They can only be predictive since major differences exist between the small mammals and humans.

Acknowledgements: The authors thankfully acknowledge the financial support of the University of Cape Town and the Medical Research Council of South Africa. We appreciate the staff of Abia State University Uturu for their fruitful help in the collection and identification of the plant species.

REFERENCES

Abosi, A.O., Raseroka, B.H., (2003): *In vivo* antimalarial activity of *Vernonia amygdalina*. *Br J. Med. Sci*., 60: 89-91.

Alves, T.M., Nagem, T.J., de Carvalho, L.H., Krettli, A.U., Zani, C.L., (1997): Antiplasmodial triterpene from *Vernonia brasliana*. *Planta Med*., 63: 554-555.

Ajebesone, P.E., Aina, J.O., (2004): Potential African substitutes for hops in tropical beer brewing. *J. Food Tech. Afr.*, 9: 13-16.

Blanco, M.M., Costa, C.A.R.A., Freire, A.O., Santos Jr, J.G., Costa, M., (2009): Neurobehavioural effect of essential oil of *Cymbopogon citratus* in mice. *Phytomedicine*, 16: 265-270.

Carlini, E.A., Contar, J.D.P., Silva-Filho A.R., da Silveira-Filho, N.G., Frochtengarten, M.L., Bueno, O.F., (1986): Pharmacology of lemon grass (*Cymbopogon citratus* Staph). Effects of teas prepared from the leaves on laboratory animals. *J. Ethnopharmacol*., 17:37-64.

Cheel, J., Theoduloz, C., Rodriguez, J., Schmeda-Hirshmann, G., (2005): Free radical scavengers and antioxidants from Lemongrass (*Cymbopogon citratus*). *J. Agric. Food Chem*., 53: 2511-2517.

Clarkson, C., Maharaj, V., Crouch, N.R., Olwem, M.G., Pillay, P., Matsabisa, G.M., Bhagwadin, N., Smith, P.J., Folb, P.I., (2004): *In vivo* antiplasmodial activity of

medicinal plants native to or naturalised in South Africa. *J. Ethnopharmacol*., 92: 177-191.

Erasto, P., Grierson, D.S., Afolayan, A.J., (2006): Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *J. Ethnopharmacol*., 106: 117-120.

Erasto, P., Grierson, D.S., Afolayan, A.J., (2007a): Antioxidant constituents in *Vernonia amygdalina* leaves. *Pharm Bio*., 45:195-199.

Erasto, P., Grierson, D.S., Afolayan, A.J., (2007b): Evaluation of antioxidant activity and fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. *J. Food Chem*., 104**:** 636-642.

Farombi, E.O., (2003): African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *Afr. J. Biotech*., 2: 662-671.

Francois, G., Passreiter, H., Woerdenbag van Looveren, M., (1996): Antiplasmodial activities and cytotoxic effects of aqueous extracts and sesquiterpene lactones from *Neutolaena lobata*. *Planta Med*., 62**:** 126-129.

Gresham, L.J., Ross, J., Izevbigie, E.B., (2008): *Vernonia amygdalina*: Anticancer activity, authentication and adulteration detection. *Int. J. Environ. Res. Pub Health*, 5**:** 342-348.

Huffman, M.A., Koshimizu, K., Ohigashi, H., (1996a): Ethnobotany and zoopharmacognosy of *Vernonia amygdalina*, a medicinal plant used by humans and chimpanzees. *Biology and Utilization*, 2: 351-360.

Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A., Igoli, N.P., (2005): Traditional medicine practice amongst the Igede people of Nigeria Part II. *Afr. J. Trad. Complem. Alternat. Med*., 2: 134-152.

Iwalewa, E.O., Iwalewa, O.J., Adeboye, J.O., (2003): Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* less leaf. *J. Ethnopharmacol*., 86: 229-234.

Iwalokun, B.A., (2008): Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. *Afr. Health Sci*., 8: 25-35.

Izevbigie, E.B., Bryant, J.L., Walker, A., (2004): A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. *Exp. Biology Medicine*, 229:163-169.

Kumaratilake, L.M., Robinson, B.S., Ferrante, A., Poulos, A., (1992): Antimalarial properties of n-3 and n-6 polyunsaturated Fatty acids: *in vitro* effects on *Plasmodium falciparum* and *in vivo* effects on *P. berghei*. *J. Clinical Investigation*, 89: 961-967.

Koshimizu, K., Ohigashi, H., Huffman, M.A., (1994): Use of *Vernonia amygdalina* by wild Chimpanzee: possible roles of its bitter and related constituents. *Physiology and Behaviour.*, 56: 1209-12016.

Masaba, S.C., (2000): The antimalarial activity of *Vernonia amygdalina* Del. Transac Royal Soc. *Trop. Med. Hyg*., 94: 694-695.

Medical Research Council (South Africa), (2004): Guidelines on ethics for medical research: Use of animals in research and training. Book 3. http://www.sahealthinfo.org/ethics/book3.htm

Melo, S,de F, Soares, S.F., da Costa, R.F, da Silva, C.R., de Oliveira, M.B.N., Bezerra, R.J.A.C., de-Araújo, Filho, M.B., (2001): Effect of the *Cymbopogon citratus*, *Maytenus ilicifolia* and *Baccharis genistelloides* extracts against the stannous chloride oxidative damage in *Escherichia coli*. *Mutat. Res*., 496: 33-38.

Oleszek, W., Igile, G., Burda, S., Jurzysta, M., (1995): Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *J. Agric. Food Chem*., 4: 2162-2166.

Onabanjo, A.O., Agbaje, E.O., Odusote, O.O,. (1993): Effects of aqueous extracts of *Cymbopogon citratus* in Malaria. *J. Proto Res*., 3: 40-45.

Orrego, R., Leiva, E., Cheel, Jose.,(2009): Inhibitory effect of three *C*-glycosyflavonoids from *Cymbopogon citratus* (Lemon grass) on human low density lipoprotein oxidation. *Molecules*, 14: 3906-3913.

Patrick, E.E., Item, J.A., Eyong, U.E.,Godwin, E.E., (2008): The Antidiabetic Efficacy of Combined Extracts from Two Continental Plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf). Am. J Biochem Biotechnol., 4: 239-244.

Pedersen, M.M., Chukwujekwu, J.C., Lategan, C.A., van Staden, J., Smith, P.J., Staerk, D., (2009): Antimalarial sesquiterpene lactones from *Distephanus angulifolius*. *Phytochemistry,* 70: 601–607.

Peters, W., Robinson, B.L., Tovey, G., Rossier, J.C., Jefford, C.W., (1993): The chemotherapy of rodent malaria. L. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 3: Observations on Fenzan-50F', a difluorinated 3,3´-spirocyclopentane 1,2,4-trioxane. *Annals Trop. Med. Parasitol*., 87: 111-123.

Phillipson, J.D., Wright, C.W., Kirby, G.C., (1993): Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases. International Symposium of the Phytochemical Society of Europe, Abstract Book University of Lausanne, Lausanne, Switzerland p3.

Suhr, K.I., Nielsen, P.V., (2003): Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *J. Appl Microbiol*., 94: 665-674.

Tchoumbougnang, F., Zollo, P.H., Dagne, E., Mekonnen, Y., (2005): *In vivo* antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* on mice infected with *Plasmodium berghei*. *Planta Med*., 71: 20-23.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., de Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totte, J., Vlientinck, A.J., (2004): *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *J. Ethnopharmacol*., 93: 27-32.

Topcu, G., Aydogmus, Z., Imre, S., Göeren, A.C., Pezzuto, J.M.; Clement, J. A.; Kingston, D.G.I., (2003): Brominated sesquiterpenes from the red alga *Laurencia obtusa*. *J Nat Prod* 66: 1505-1508.

Tortoriello, J, Romero, O., (1992): Plants used by Mexican traditional medicine with presumable sedative properties: an ethnobotanical approach. *Archives Med. Res*., 23: 111-116.

Tiwari, A.K., Rao, J.M., (2002): Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci*., 83: 30-37.

Viana, G.S.B, Vale, T.G., Pinho, R.S.N., Matos, F.J.A., (2000): Antinociceptive effect of the essential oil from *Cymbopogon citratus* in mice. *J. Ethnopharmacol*., 70: 323-327.

White, N.J., (1998): Preventing antimalarial drug resistance through combinations. Drug Resistance Updates 1: 3-9.

Wilkinson, J.M., Cavanagh, M.A., (2005): Antibacterial activity of essential oils from Australian native plants. *Phytother. Res*., 19: 643-646.

World Health Organisation 2001: Antimalarial drug combination therapy: report of a WHO technical consultation, April 4-5 Geneva. WHO/CDS/RBM/2001.35

World Health Organisation., (2006): Facts on ACTs (artemisinin-based combination therapies). January 2006 update. (Accessed 10th June 2010) http://www.rbm.who.int/cmc_upload/0/000/015/364/RBMInfosheet_9.htm

Table- 1: Percentage parasite inhibition of *C. citratus* (DCM) combined with *V. amygdalina* (DCM) *in vivo*.

Extract/drug administered (dose mg/kg)	Parasite growth inhibition on Day 3 of treatment (%)	Parasite growth inhibition on day 1 post treatment (%)
Chloroquine(10 mg/kg)	71	70
C. citratus + *V. amygdalina* (400 mg/kg each)	85	87
C. citratus + *V. amygdalina* (600 mg/kg each)	95	100

- DCM: dichloromethane

Table- 2: Weights of the groups during the combination experiments.

Days post infection	CQ (10mg/kg)	mH_2O	*C. citratus* DCM+ *V. amygdalina* DCM (400 mg/kg each)	*C. citratus* DCM+ *V. amygdalina* DCM (600 mg/kg each)
0	22.01±1.71	23.50±1.31	24.15±1.87	23.65±1.81
1	21.98±1.24	22.39±0.73	24.94±1.68	24.60±1.73
2	21.88±0.73	21.91±1.26	24.64±1.79	24.47±1.55
3	20.87±0.50	21.87±0.84	24.64±1.65	24.28±1.54
4	21.03±1.30	22.12±0.86	23.77±1.33	22.28±0.91
5	20.93±1.80	21.23±0.59	22.92±1.40	21.6±1.46
8	18.47±1.18	19.7±0.87	19.36±2.41	20.95±2.14
10	18.35±0.64	---------	18.73±2.35	22.9±1.63
11	17.8	----------	18.3±2.61	23.23±1.61
14	---------	----------	----------	22.89±1.11
16	---------	---------	---------	23.59±0.99
18	---------	---------	---------	23.01±0.81
23	----------	---------	---------	24.11±0.86
28	----------	---------	---------	25.17±0.69
32	---------	----------	---------	25.91±0.56
35	----------	--------	---------	25.86±0.82
38	----------	----------	---------	25.87±0.48
37	----------	----------	---------	26.62±0.55

- CQ: chloroquine; DCM: dichloromethane; mH_2O: millipore water