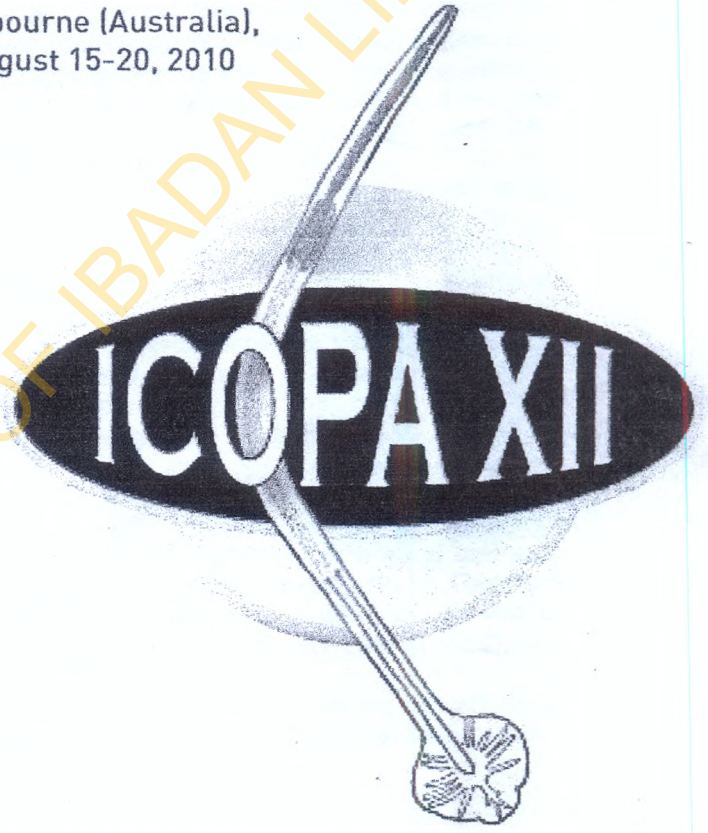
MEDIMOND - Monduzzi Editore International Proceedings Divisi

XII International Congress of Parasitology

ICOPA

Melbourne (Australia),
August 15-20, 2010



MEDIMOND
INTERNATIONAL PROCEEDINGS

In-vitro antimalarial activities of Withania somnifera, Gymnema sylvestre, Ocimum gratisimum and Cajanus cajan.

Morenikeji O.A[1], Pillai C.R[2], Valecha N[2] and Dash A.P[2]

[1]Department of Zoology, University of Ibadan, Nigeria.
[2] National Institute of Malaria Research, Delhi, India.

Summary

A study was carried out to determine the efficacy of four plants, *Withania somnifera (WS), Gymnema sylvestre (GS), Ocimum gratisimum (OG)* and *Cajanus cajan (CC)* traditionally used in the treatment of malaria in Nigeria and India. Ethanolic extracts of these plants were tested in vitro on choloroquine sensitive (MRC-pf-2) and resistant (MRC-pf-303) strains of *Plasmodium falciparum* for their anti-malarial activity. The IC_{50} values of these plants were calculated and found to be OG (IC_{50} = 23.48 µg/ ml), WS (IC_{50} = 45.24 µg/ ml), GS (IC_{50} = 45.25 µg/ ml) for the pf sensitive strain and CC (IC_{50} = 47.52 µg/ ml) in the pf resistant strain. Others, GS IC_{50} = 69.70 µg/ ml (resistant strain), CC IC_{50} = 72.31µg/ ml (sensitive strain), WS IC_{50} = 88.30 µg/ ml (resistant strain), OG IC_{50} = 121.76µg/ ml (resistant strain). Higher concentrations caused total inhibition of the parasite at the ring stage. *Ocimum gratisimum* showed the most significant antimalarial effect on the parasites sensitive strain. Results lend some credence to the use of medicinal plants in the treatment of malaria and the need to study the active constituents of effective ones.

Keywords: In-vitro studies, *Plasmodium falciparum*, Medicinal plants.

Introduction

Malaria remains a major cause of morbidity and mortality in tropical and sub-tropical regions of the world, despite decades of malaria control efforts. There are approximately 300-500 million clinical cases and about one million deaths due to malaria globally, and Africa south of the Sahara accounts for over 90% of the disease burden (Snow *et al*, 2005)

Spread of multidrug-resistant strains of Plasmodium and the adverse side effects of the existing anti-malarial drugs have necessitated the search for novel, well-tolerated and more efficient antimalarial drugs.

Interest in plants as new anti-malarials has been stimulated by the isolation of artemisinin, a highly active compound against drug-resistant *P. falciparum* from *Artemisia annua*. So also about 80% of the world's population use medicinal plants for primary health care (Cordell, 1995), and traditional medicines are the "only health resource available to about 60% of the world population" (Taylor *et al*., 2001). Medicinal plants have proven their efficiency for their "safety, efficacy, cultural acceptability and lesser side effects" (Kamboj, 2000).

This study investigates the antimalarial activities of herbal leaves used in traditional medicine for malaria treatment in Nigeria and India.

Materials and methods

Herbal plants collected in Nigeria were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, *Ocimum gratisimum* (FHL./108338) and *Cajanus cajan* (FHL./108337).

Gymnema sylvestre and *Withania sommifera* with taxonomic serial numbers TSN 506007 and 505824 respectively, as per the Integrated Taxonomic Information System (ITIS) were collected in India.

50% ethanol extract of *O.gratisimum* and *C.cajan* powdered materials was prepared by adding 50g of each powder to 50% ethanol. Ethanol extraction was carried out in a Soxlet apparatus maintaining boiling for five rounds. The extract was then filtered out and the filtrate concentrated over a water bath to dryness.

100% ethanol extract of *Gymnema sylvestre* and *Withania sommefera* was prepared by taking 20gm of the powdered materials in separate conical flasks.200ml of ethanol was added to each flask ,shaken well and kept for 24hrs.The supernatant was collected and again same quantity of ethanol was added to each flask. This process was repeated thrice. Then the combined ethanol extract was evaporated to dryness under reduced pressure to get the crude extract.

P. falciparum laboratory lines, MRC-PF-303(Resistant strain) and MRC-PF 2(Sensitive strain) used were cryopreserved in liquid nitrogen at the Parasite Bank, Malaria Research Centre, Delhi and maintained in culture with A^+ blood group erythrocytes at 5% hematocrit in RPMI 1640 medium (GIBCO BRL, Life Technologies Inc. USA) supplemented with 10% AB^+ human serum, gentamicin (10 µg ml^{-1}), 25 mM sodium bicarbonate and 25 mM HEPES.

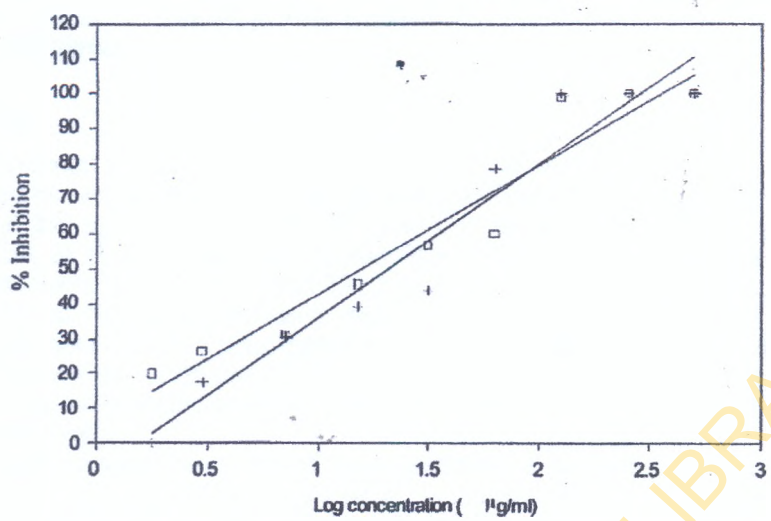
The culture was synchronized using 5% aqueous solution of sorbitol. Degenerated stages were removed by centrifuging for 5minutes at 1500rpm.The supernatant was discarded and the pellet was washed twice with incomplete media.

Parasitaemia was adjusted to about 1% for assay by diluting with fresh washed RBCs. The material to be tested was dissolved in 100µl of DMSO.The stock solution was diluted in RPMI-1640 incomplete medium to obtain different concentrations.

Different concentrations of extract were dispensed in 96 well plate in duplicate. The first well in all the rows were without any drug and considered to be control. The synchronized parasites were inoculated to all the wells including control wells to get a final concentration of 5% heamatocrit.

The plates were incubated at 37° C for 24-30 hrs depending on the maturation of the schizont and smears were prepared from all the wells. The smear was stained with JSB stain and number of schizonts were counted per 200 asexual stage parasites.

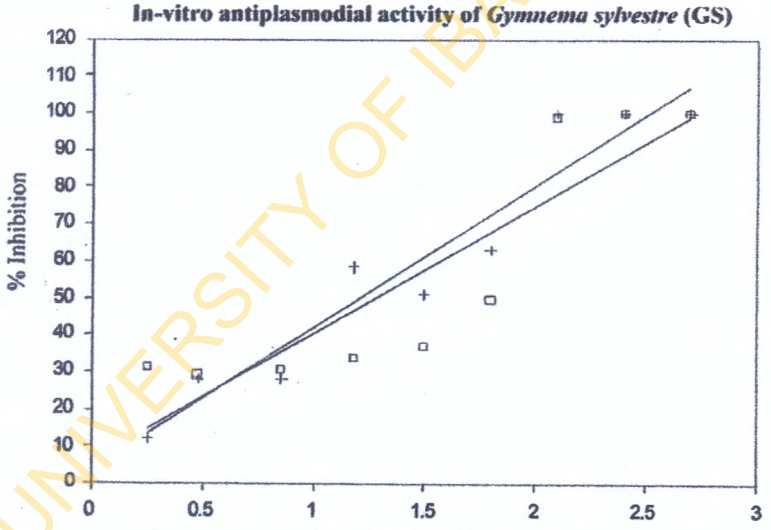
In-vitro antiplasmodial activity of *Cajanus cajan* (CC)



+ Sensitive 44.247x - 8.5544 R ² = 0.9441
□ Resistant 37.008x + 5.2493 R ² = 0.9345

Susceptible at 50% = 72.308
Resistant at 50% = 47.519

In-vitro antiplasmodial activity of *Gymnema sylvestre* (GS)



+ Sensitive 18.356x + 3.6252 R ² = 0.9233
□ Resistant 1.228x + 6.2391 R ² = 0.7886

Susceptible at 50% = 45.253
Resistant at 50% =69.709

Statistical Analysis

The 50% inhibitory concentration (IC50), defined as the drug concentration at which 50% of the parasite growth is inhibited, compared with drug-free control wells were calculated.

Results

Maximum growth inhibition (97.92% in chloroquine-sensitive and 99.05% in chloroquine-resistant strains) was obtained with OG at a concentration of 125 µg/ ml and 250 µg/ ml respectively. Whereas CC showed its maximum inhibitory effect (78.41% in chloroquine-sensitive and 98.60% in chloroquine-resistant strains at a concentration of 62.5 µg/ml and 250 µg/ml respectively. Maximum growth inhibition (62.98% in chloroquine-sensitive and 98.90% in chloroquine-resistant strains) was obtained with GS at a concentration of 62.5 µg/ ml and 125 µg/ ml respectively. WS showed its maximum inhibitory effect (87.69 % in chloroquine-sensitive and 73.92% in chloroquine-resistant strains at a concentration of 125 µg/ml and 250 µg/ ml respectively.

Higher concentrations caused total inhibition of the parasite at the ring stage.

The IC_{50} values of these plants were calculated and found to be lowest in the sensitive strain of OG(IC_{50} = 23.48 µg/ ml), WS(IC_{50} = 45.24 µg/ ml), GS(IC_{50} = 45.25 µg/ ml) and the resistant strain of CC (IC_{50} = 47.52 µg/ ml).

Others, IC_{50} = 69.70 µg/ ml(resistant strain, GS), IC_{50} = 72.31µg/ ml(sensitive strain, CC), IC_{50} = 88.30 µg/ ml(resistant strain, WS), IC_{50} = 121.76µg/ ml(resistant strain, OG) (Figures 1- 4).

Discussion

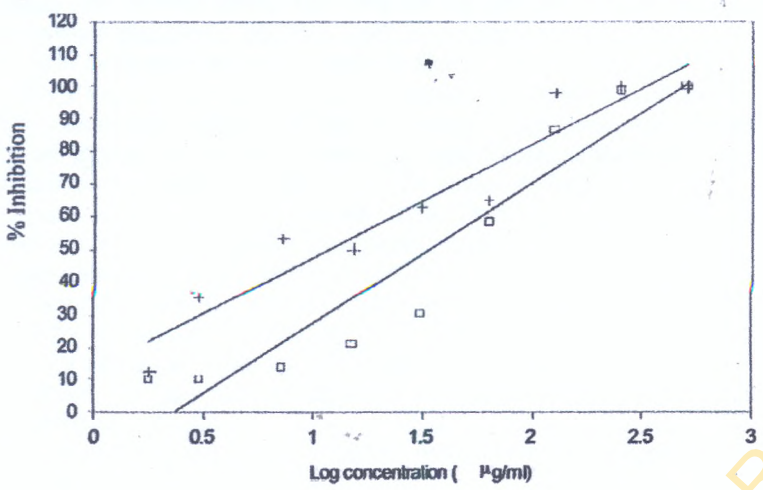
The most active extract was obtained from *OG* (Linn) family Labiaceae, a herbaceous plant, that showed appreciable inhibition of the parasites at all the concentrations used in the study. The inhibition shown by *OG* can be said to be remarkable because the plant is usually boiled with a mixture of certain other plants in Nigeria for prophylaxis or traditional chemotherapy of malaria. Occasionally, a few people take it alone.

In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy(Osifo, 1992), high fever and diarrhea (Oliver, 1980), whilst in the savannah areas decoctions of the leaves are used to treat mental illness. The leaves of the plant are also used as a condiment in cooking.

OG is also used in tropical Asia especially India and South America in traditional medicine (Corrêa, 1926).

Herb sellers in Nigeria use the leaves of CC in the treatment of malaria (Aiyeloja and Bello, 2008). *CC* is a perennial member of the family Fabaceae. Other common names are pigeon pea, arhar, Congo pea, Gungo pea, Gunga pea, and no-eye pea. Some herbal practitioners are of the opinion that it diminishes the swelling of internal organs like stomach, liver and intestines and it helps in reducing wound or cancer of these organs. In Brazilian herbal medicine, the leaves are infused for cough, fevers and ulcers among other medicinal uses.In Chinese folk medicine pigeon pea leaves

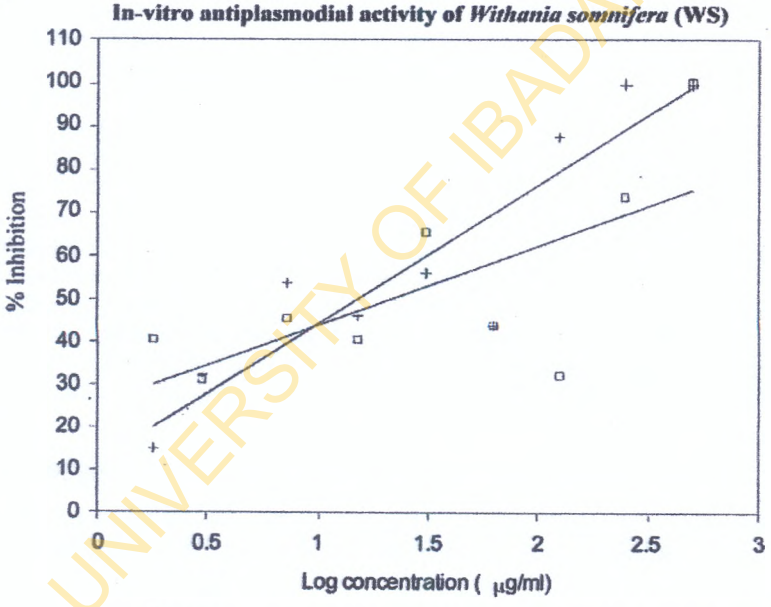
In-vitro antiplasmodial activity of *Ocimum gratisimum* (OG)



+ Sensitive 4.649x + 13.226 R^2 = 0.9256 Sensitive at 50% = 23.488

□ Resistant 13.248x - 15.805 R^2 = 0.9065 Resistant at 50% = 121.763

In-vitro antiplasmodial activity of *Withania somnifera* (WS)



+ Sensitive y = 32.423x + 11.774 R^2 = 0.843 Sensitive at 50% = 45.237

□ Resistant y = 18.434x + 25.471 R^2 = 0.4728 Resistant at 50% = 88.303

are used to staunch blood, as an analgesic and to kill parasites (Zu Yg *et al*, 2006). In Peru, the leaves are prepared in a infusion for anemia, hepatitis, diabetes, urinary infections, and yellow fever. The flowers are prepared in an infusion for dysentery, and menstrual disorders; and the seeds are infused to use as a diuretic.

GS is a herb native to the tropical forests of southern and central India where it has been used as a naturopathic treatment for diabetes for nearly two millennia. Its major bioactive constituents are a group of oleanane type triterpenoid saponins known as gymnemic acids. Gymnemic acids have antidiabetic, antisweetener and anti-inflammatory activities and it is still being studied.

WS, also known as Ashwagandha, Indian ginseng, winter cherry, is a plant in Solanaceae or nightshade family. This stout shrub grows prolifically in India, Pakistan, Sri Lanka and Bangladesh. In Ayurveda it is considered a rasanaya herb, which works on a nonspecific basis to increase health and longevity. It also has sedating properties and used for the Ayurvedic system as aphrodisiacs, diuretics and for treating memory loss. It is used in the treatment of fever and cold in tribal areas of India.

The results of this study lend some credence to the use of traditional medicine in the treatment of malaria. There is need for research to study their active consituents, to establish their efficacy and to determine their potentials as sources of new antimalarial drugs.

References

AIYELOJA A.A AND BELLO O.A(2008). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. Educational Research and Review Vol. 1 (1) 16-22.

CORDELL G.A (1995). Changing strategies in natural products chemistry. Phytochemistry 40: 1585–1612.

CORRÊA, M. P (1926), Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas. Rio de Janeiro: Imprensa Nacional.

KAMBOJ V.P (2000). Herb. Med. Curr. Sci. 78(1): 35-39.

OLIVER, B. (1980). Medicinal Plants in Nigeria. Published by Nigerian College of Arts, Science and Technology. Ibadan, Pp. 90-94.

OSIFO, N.G. (1992). A System of Traditional Health Care. VoL2. Pp. 56.

SNOW R.W, GUERRA C.A, ABDISALAN M, MYINT H.Y, HAY S.I (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature,* 343:214-217

TAYLOR J.L.S, RABE T, MCGAW L.J, JÄGER A.K, VAN STADEN J (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 34: 23–37

ZU YG FU YJ, LIU W, HOU CL(2006). Simultaneous determination of four flavonoids in Pigeonpea [Cajanus cajan (L.) Millsp.] leaves using RP-LC-DAD. Chromatographia, 63:499.

Acknowledgement

We are grateful to Mrs Sangeeta Arora for laboratory assistance, Mr Joghinda, Mr Semwal and other staff members of the Parasite Bank, NIMR, Delhi, India.

This study was funded by TWAS-DBT Fellowship.