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Silver Spring, Maryland 20902

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13. ABSTRACT ***(Maximum 200 Words)***

During the reporting period, 206 plant samples were submitted for antimalarial screen out of which 19 plants showed activities against D-6 and W-2 strains of *Plasmodium falciparum*. In our priority list of potential antimalarial candidates, the following plants were bulk extracted: *Picralima nitida, Spathodea campanulata, Uvaria chamae, Cryptolepis sanguinolenta, Glossocalyx brevipes, Cleistopholis patens, Leidobotrys staudtii, Pachypodanthium staudtii, Odyendyea gabonensis* and *Uapaca paludosa.* Eleven compounds were isolated from two plants - *Penianthus longifolius* and *Homalium letestui* that showed antimalarial and antitrypanosomal activities. Additional eight pure compounds were isolated from extracts of *Glossocalyx brevipes* that showed antitrypanocidal and antileishmanial activities. Fifty-two extracts were screened for antimicrobial activities out of which 60% showed very significant antibacterial activity and 7 of the extracts showed antifungal activity. Four antimicrobial compounds were also isolated from *Peucedanum zenkeri* and *Araliopsis tabuoensis.* Twenty-one compounds were synthesized using cryptolepine as template to enhance their antimalarial and oral activity. Extracts were also processed for testing against HIV, cystic fibrosis, cancer and CNS. In collaboration with the Environmental Law Institute, Washington DC we are examining options for new approaches to the valuation of natural resources. A re-census of the 50ha Korup biodiversity plot was conducted with the Smithsonian Institution.

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M. Manning IV 10-18-02

PI - Signature **Date**

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INTRODUCTION:

The African ICBG, in general emphasizes three major goals: evaluation of rainforest plants from Nigeria and Cameroon as cures for parasitic diseases; research on forest dynamics to understand the effects of sustainable harvesting and cultivation of important medicinal plants; training of Cameroonians and Nigerians in natural products chemistry and tropical ecology.

The ICBG project, jointly sponsored by the U.S. National Institutes of Health, the National Science Foundation and the U.S. Department of Agriculture has the main focal point of establishing an integrated program for the discovery of biologically active plants for drug development and biodiversity conservation, while ensuring that source countries derive maximum benefits for their biological resources and their intellectual contribution. BDCP facilitates the drug discovery component of the ICBG and therefore serves as a link between the drug discovery part of the program, the biodiversity conservation component and the economic development projects. The aims of the Associate Programs administered by BDCP are:

1 To conduct ethnobiological inventory of plants in the selected study areas;
2. To guide the ICBG in its plant selection and collection strategies for drug discovery. Samples identified from ethnobiological inventory will be collected from biodiversity plots and from wild flora and screened for possible biological activity.
3. To perform phytochemistry and preliminary bioassays on selected plants.
4. To perform plant extraction, bioassay-guided isolation and structural elucidation, with research, training and infrastructure development being important components of each operation.
5. To maintain and expand the database on African medicinal plants, which includes information on local names, traditional, uses, floristic data, possible constituents, conservation status, agronomic data and economic value. This involved the re-structuring and expansion of the existing AfricMed database to include data from other Associate Programs. This Computerized Information System of African Medicinal Plants (CISAMAP) will be linked to other regional databases.
6. To conduct a socio-economic value assessment of the biological resources in the study area which seeks to:
    - I) highlight the non-commercial value of forest products within the cultural/ religious context;
    - II) quantify the economic value of biological resources for comparison with other land use options;
    - III) place in priority order the production and marketing of biological resources in local markets to provide income for local residents;
    - IV) provide baseline agronomic data for the formulation of a sustainable management plan for the forest resources; and
    - V) train local natural resource managers and users at the National and Community levels to conduct economic and market research which will integrate the connection between conservation and development. The ICBG may organize rural farmers to cultivate, in fallow areas, certain plants of potential therapeutic value;

7. Assist in capacity building of West African scientists in the areas of ethnobiology, inventory and research management. Formal training will be organized in ethnobiological methods and field taxonomy and economic value assessment for local communities.

BODY:

KEY RESEARCH ACOMPLISHMENTS:

1.1 PHYTOCHEMISTRY & PRELIMINARY BIOASSAYS

Specific Aims:
1. Evaluate the biological activity of plants used in African ethnomedicine.
2. Isolate and characterize the bioactive constituents of these plants.
3. Perform bulk extraction and fractionation of plant material for *in vivo* assays.
4. Optimize lead compounds with significant potential for human health in Africa.
5. Provide training for African scientists at all levels of drug development.

Institutions:
University of Dschang, Cameroon.
International Center for Ethnomedicine and Drug Development, Nsukka, Nigeria.
University of Buea, Cameroon
University of Minnesota, Minneapolis, Minnesota.
University of Mississippi

Progress Report:
This report summarizes the activities carried out by collaborating scientists at the participating institutions named above. All planned activities were actively pursued during the period covered by this report. Details are provided below:

1.1.1 Phytochemical Investigations.

A. Extractions:
As envisaged, a large number of small-scale and bulk extractions were carried out during the period covered by this report. Small-scale extractions were performed to provide new candidates for screening and to increase the size of the extract library, while bulk extractions were carried out on previously identified active extracts to provide material for isolation of active constituents and for in vivo bioassays.

For small-scale extractions, the following plant parts were used wherever appropriate: stem, aerial parts, stem bark, leaves, fruit, seed and rhizome. Each sample was divided into two parts for extraction with methanol and methylene chloride following a standard procedure. A list of these extracts is provided in Table 1.

TABLE 1 List of Plants Extracted during the reporting Period.

Methylene chloride extracts:

	Plant	**Plant part**
1)	*Paropsia guineensis*	leaves
2)	*Synedrella nodiflora*	whole plant.
3)	*Sterculia tragacantha*	aerial parts
4)	*Momordica charantha*	leaves
5)	*Mussaenda elegans*	leaves
6)	*Macrosphyla longistyla*	leaves
7)	*Chasmenthera dependens*	leaves
8)	*Scoparia dulcis*	whole plant.
9)	*Eclipta prostrate*	aerial parts.
10)	*Morinda lucida*	leaves
11)	*Erythrina senegalensis*	stem bark
12)	*Brenania breyi*	leaves
13)	*Schumaniophyton problematic*	leaves
14)	*Osbeckie sp*	aerial parts.
15)	*Chasmanthra dependens*	stem bark.
16)	*Hoslundia opposta*	stem
17)	*Dracaena mannii*	stem
18)	*Paropsia guineensis*	stem
19)	*Ficus exasperata*	stem.
20)	*Crassocephalum sp*	whole plant.
21)	*Olax subscorpoides*	leaves.
22)	*Ehretia cymosa*	leaves.
23)	*Ehretia cymosa*	stem.
24)	*Berlina grandifolia*	leaves.
25)	*Ritchea capparoides*	roots.
26)	*Sphenocentrum jollyanum*	roots.
27)	*Crescentia cujeta*	fruit juice.
28)	*Ritchea capparoides*	roots
29)	*Platycerium bifurcatum*	leaves.
30)	*Hannoa klainiana*	leaves.
31)	*Voacanga africana*	stem.
32)	*Petersianthus macrocarpus*	leaves.
33)	*Tabernataemontana pachysiphon*	leaves
34)	*Funtumia elastica*	leaves.
35)	*Pterygota macrocarpa*	leaves.
36)	*Jatropha gossypiifolia*	leaves.

Methanol extracts:

37)	*Momordica charantha*	aerial parts.

38)	*Paropsia guineensis*	leaves
39)	*Synedrella nodiflora*	whole plant.
40)	*Sterculia trangacantha*	leaves.
41)	*Hoslundia oppoita*	stem.
42)	*Paropsia guineensis*	stem.
43)	*Dracaena mannii*	stem
44)	*Berlina grandifolia*	leaves.
45)	*Ritchea capparoides*	roots.
46)	*Mormodica charantha*	fruits.
47)	*Sphenocentrum jollyanum*	roots.
48)	*Ritchea capparoides*	roots.
49)	*Platycerium bifurcatum*	leaves.
50)	*Tabernaemontana pachysiphon*	leaves.
51)	*Ficus exasperata*	stem.
52)	*Crassocephalum sp.*	Whole plant.
53)	*Olax subscorpoides*	leaves.
54)	*Ehretia cymosa*	leaves.
55)	*Ehretia cymosa*	stem.
56)	*Funtumia elastica*	leaves.
57)	*Pterygota macrocarpa*	leaves.
58)	*Jatropha gossypiifolia*	leaves.
59)	*Garcina kola*	seeds.
60)	*Zingiber officiales*	rhizomes.
61)	*Ocimum gratissimum*	leaves.
62)	*Afromamum melegueta*	seeds.

Bulk extractions were carried out for the following plants: *Picralima nitida, Enantia chlorantha, Spathodea campanulata, Synclisia scarbrida, Uvaria chamae, Cryptolepis sanguinolenta, Glossocalyx brevipes, Cleistopholis patens, Leidobotrys staudtii, Pachypodanthium staudtii, Odyendyea gabonensis* and *Uapaca paludosa*.

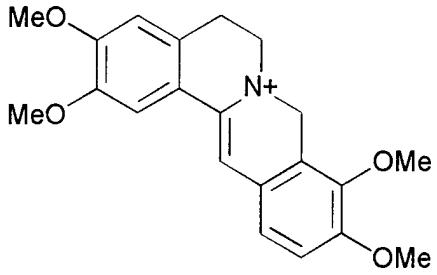
Moreover, previously identified extracts with activity against malaria such as *Combretum dulchipelatum* were fractionated and the fractions were submitted for biological testing in a bid to localize the active constituents. Other plants in this category include *Dracaena mannii* (CNS and antileishmanial activity), *Cassytha filiformis* (CNS activity), *Triumfetta tomentosa* (CNS activity), *Scoparia dulcis* (CNS) and *Combretum dulchipelatum* (antimicrobial). Detailed phytochemical investigations are proceeding on these compounds.

B. Isolation & Characterization of Plant Secondary Metabolites:
Bioassay-guided fractionation of above mentioned previously identified leads led to the isolation of several metabolites, whose identities have been determined in a number of cases. Further biological evaluation of the isolated compounds has confirmed the identities of the active constituents of these extracts.

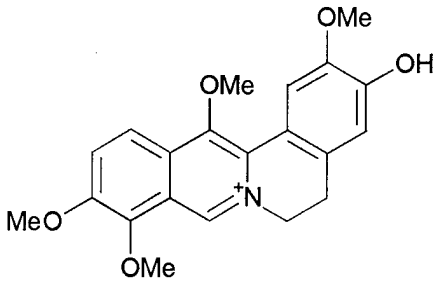
Penianthus longifolius (Menispermaceae)

The CH2Cl2-MeOH (1:1) extract of the stem bark is highly active against *P. falciparum* (D6: IC50 = 350.066 ng/ml; W2: IC50 = 284.377 ng/ml). Repeated column chromatography of the extract led to the isolation of ten pure compounds designated PL1-PL10. PL1 and PL3 were subsequently identified as fats, while PL4 was identified as b-sitosterol glycoside. PL5 and PL7 which crystallized in different shapes in the same solvent are two different crystalline forms of the same compound – palmatine - PL6 and PL8 were identified as jatrorrhizine and vibroquercitol, respectively, while PL9 was found to be sucrose. PL10, a component isolated from the alkaloid fraction of the extract, was identified as N-methylcoridinium. The biological activities of the secondary metabolites are presented in Table 2 below.

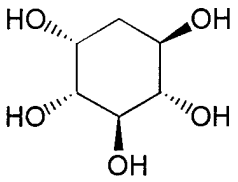
Figure 1: Secondary Metabolites Isolated from *P. longifolius*.



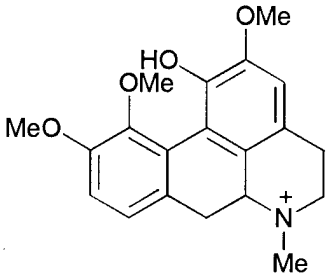
PL5=Palmatine



PL6: Jatrorrhizine



PL8: Viboquercitol



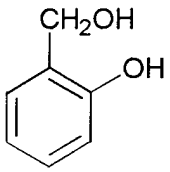
PL10 : ammonium salt of N-methyl-corydine

Table 2: Antiplasmodial activity of Compounds (PL1-7) isolated from *Penianthus longifolius*

	IC50 ng/mL	
	D-6	**W-2**
PL1	> 5000	> 5000
PL2	> 5000	> 5000
PL3	> 5000	> 5000
PL4	> 5000	> 5000
PL5	24.4215	37.3638
PL6	67.4211	142.4276
PL7	26.6167	37.7054

Homalium letestui (Flacourtiaceae).
Phytochemical studies were initiated on this plant because the acetone extract of the stem bark displays significant antiplasmodial activity (W2: IC50 = 2564.11 ng/ml). Repeated column chromatography of this extract, combined with other separation techniques has so far yielded six constituents designated HL1-HL6. One compound, HL3, has been identified as 2-(hydroxymethyl) phenol. Efforts are currently underway to elucidate the structures of the other constitutents.

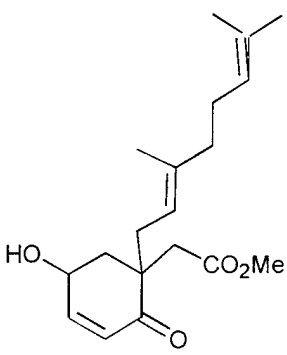
Figure 2: Structure of HL3 Structures of the compounds isolated from *Homalium letestui*.



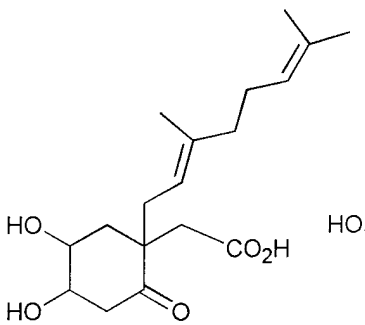
HL3

Glossocalyx brevipes (Monimiaceae)
Extracts of this plant display both antiplasmodial and antileishmanial activity. Eight pure compounds (GBF1- GBF8) were isolated from the leaf extract while three (GBE2-GBE4) were obtained from the stem bark of this plant (Figure 4). Spectroscopic analysis led to the identification of seven compounds, including three alkaloids and four homogentisic acids. All four homogentisic acids (GBF1, GBF3, GBF5 & GBF6) and one alkaloid (GBF1) were identified as new compounds. Structure elucidation of the stem bark constituents is currently underway.

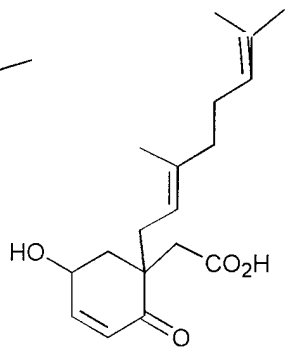
Figure 3: Structures of the compounds (GBF1-3; GBF5-8) isolated from *G. brevipes*



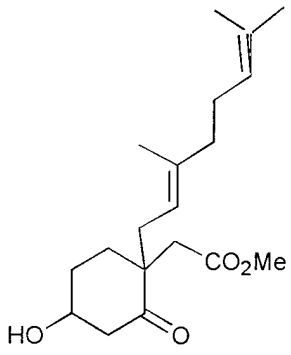
GBF1



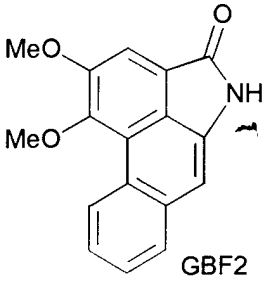
GBF3



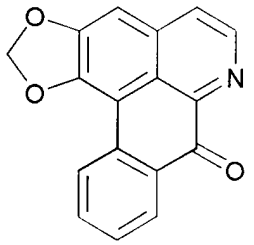
GBF5



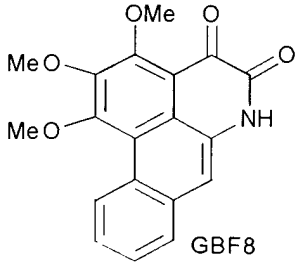
GBF6



GBF2



GBF7=Linodendrine



GBF8

As shown in Table 3 below, some of the compounds exhibited antiplasmodial activity.

	IC50 ng/mL	
	D-6	**W-2**
GBF1	702.5863	2125.7840
GBF2	> 5000	> 5000
GBF3	> 5000	> 5000
GBF5	1462.0020	2552.9440
GBE2	> 5000	> 5000
GBE3	1326.2950	2373.7100
GBE4	1164.9080	2367.5500

Xymalos monospora (Monimiaceae):
A re-examination of the alkaline extract of this plant yielded additional quantities of TZM4 and TZMHCl. Samples of these compounds have previously shown trypanocidal activity *in vitro*. The current effort was aimed at providing additional material for *in vivo* studies in animal models of trypanosomiasis.

Hyptis suaveolens:
Hyptis suaveolens is one of the plants identified as having the ability to correct the cystic fibrosis (CF) defect in the yeast two-hybrid assay. It is therefore desirable to isolate and identify the active constituents of this plant. A combination of silica gel based chromatographic techniques such as CC and Biotage Flash chromatography led to the isolation of the compound responsible for reversing the CF defect in the yeast-based screening assay. The method adopted in the isolation process is briefly described. Dried leaf (2.0 Kg) was extracted successively with petroleum ether, chloroform, methanol and water. Each extract was concentrated to dryness using the rotary evaporator. The petroleum ether extract was most active followed by the chloroform extract. About 8.20 g of the petroleum ether extract was chromatographed on a silica gel column eluted with Hexane: Ethyl acetate (9:1). A 50-ml fraction was collected and monitored by TLC using petroleum ether: ethyl acetate (3:1). Chromatographically similar fractions were pooled and assayed. Four of the 7 major fractions showed activity in the cystic fibrosis assay, with the most active fraction being Fr. 71 +72. Further fractionation of Fr. 71-72 was carried out. About 500 mg was re-chromatographed on Biotage Flash chromatography eluted with solvent system petroleum ether/ ethyl acetate (3:1). A total of 33 fractions (20 ml each) were collected and analyzed by TLC. As shown in Figure 3, seven subtractions were obtained. Fractions 1-6 gave the highest yield of 96.5 mg. Thin layer chromatograms showed that fractions 7-18 contained a single component. The isolated compound has been submitted for further biological testing and structure elucidation is also underway.

Fig 3 Bioassay-guided fractionation Scheme of Petroleum ether extracts of *Hyptis suaveolens*

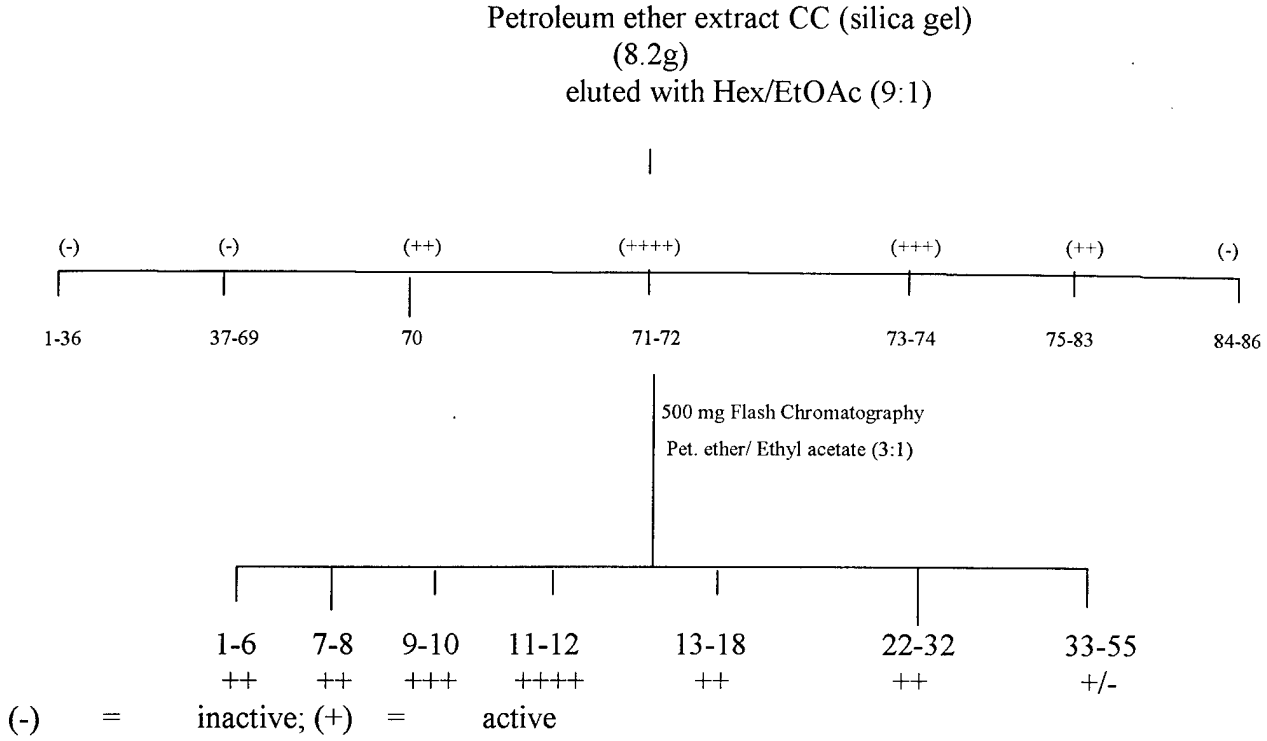
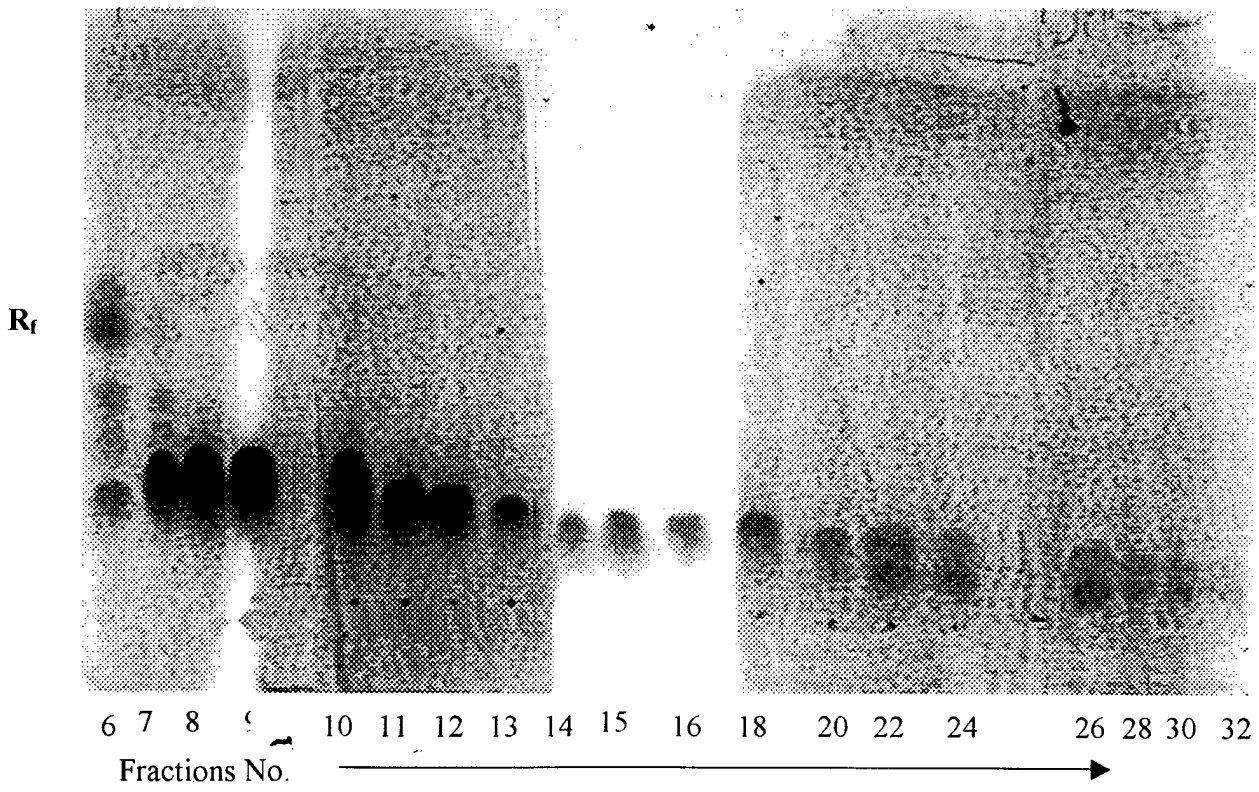


Fig 4: Chromatographic Separation of the Petroleum ether extract of *Hyptis suavelonsis*

TLC Analysis of Flash the Chromatographic Separation



The best solvent system for this TLC separation was Petroleum ether: ethyl acetate (3:1)
Fraction 6 showed a partial purification

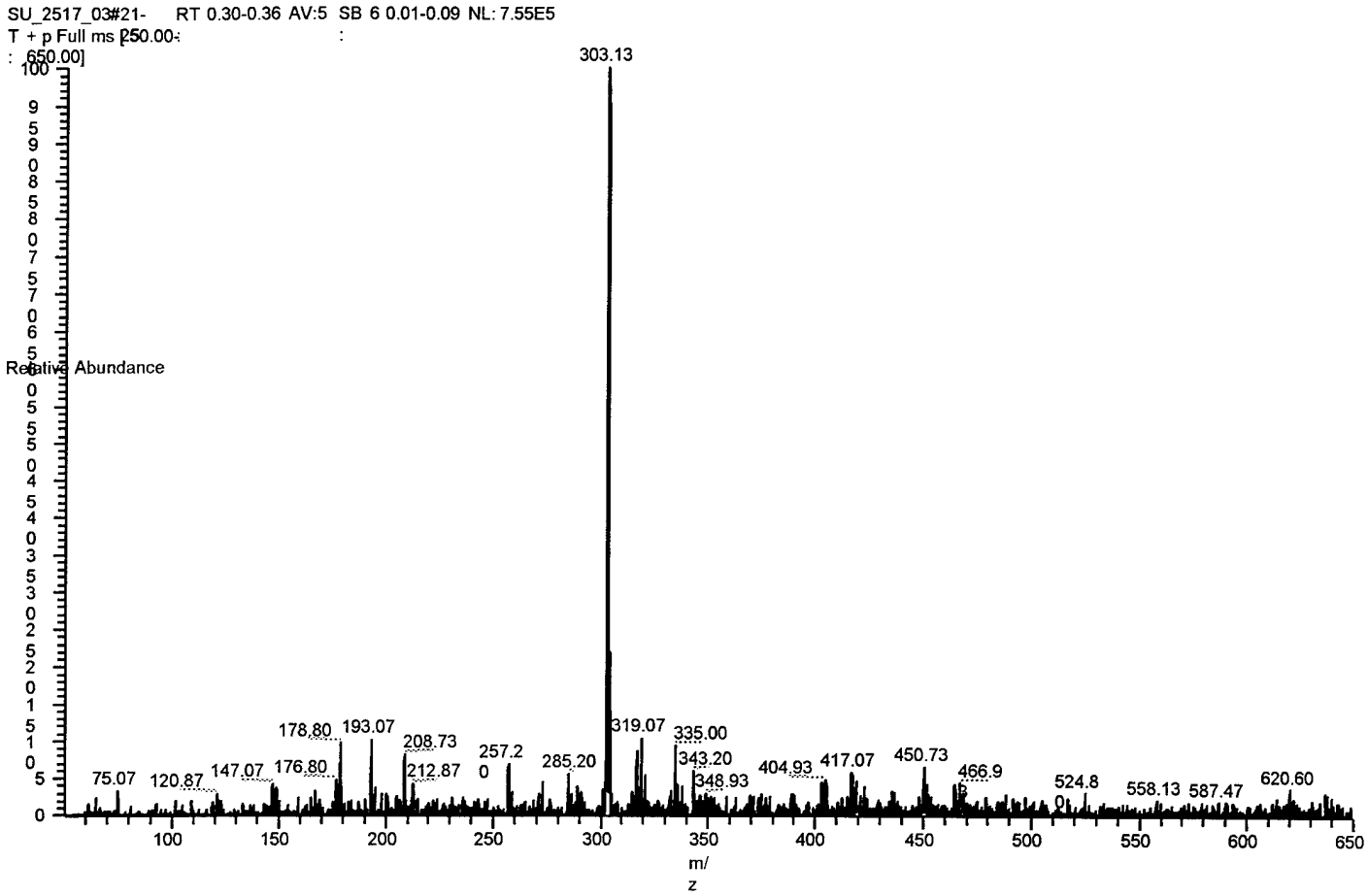


Figure 5 LC-MS of compound isolated from *Hyptis suaveolens* (above)

Trichilia spp & Thuranthus africana

Previous research by our team has shown that two constituents of these plants designated TS2 and PTA4 can correct the cystic fibrosis defect reconstruct in yeast. Additional quantities of these compounds were thus prepared for further examination of these promising compounds.

Dracaena mannii

Previously, we found that the fruit pulp extract of this compound displays significant antileishmanial activity. The extract was also found to bind monoamine transporters (see our 2000/2001 Progress Report). Our subsequent work is thus aimed at isolating and identifying the secondary metabolites that are responsible for the observed activities. To this end, the first batch of bulk extracted material was used for dereplication of antileishmanial compounds. The methanol extract containing the highest concentration of saponins was fractionated by a combination of Sepahdex LH20, lobar and preparative TLC to yield mannispirostan A- a known antileishmanial compound. Further work on this plant is on going at the University of Buea with a focus on CNS activity.

Isolation of Alstonine: Search for Alternative Sources.

Previously, we have shown that alstonine, an indole alkaloid originally isolated from *Alstonia boonei*, is the active constituent of a Nigerian medicinal plant used for the treatment of schizophrenia. Recent studies by our collaborators reveal that alstonine is an atypical antipsychotic which displays a unique pharmacological profile (Elizabetsky *et al.*, 1998). This alkaloid may thus represent a useful lead for a new class of antipsychotics agents. Therefore, we have launched a

detailed investigation of this compound. The current study was undertaken to identify alternative sources of alstonine for use in current and future pharmacological investigations.

Working quantities of alstonine were isolated from the pericarp of *Picralima nitida* by a combination of column chromatography and preparative TLC. The methylene chloride extract of the pericarp of *Picralima nitida* was fractionated on silica gel 60 column. By means of comparative TLC, the fractions containing alstonine and the pure alstonine standard were matched leading to the identification and subsequent isolation of the alstonine from the pericap of *Picralima nitida*. By a similar procedure, alstonine was also isolated from the methanol extract of the root of *Rauwolfia vomitoria*. As a result of this study, we now have three sources of this potentially interesting compound.

1.1.2 Preliminary Bioassays

1) Brine Shrimp Cytotoxicity Screening

Thirty-three extracts were assayed during the project period. However, the results obtained were inconclusive probably because the eggs used were of questionable viability. As a result, these data have been set aside and the assays will be repeated.

2) Antimicrobial Screening

A total of 52 extracts consisting of various plant parts were screened for antimicrobial activities against four bacterial organisms (*Bacillus subtilis, Staphlococcus aureus, E. coli, and Pseudomonas aeroginosa)* and in some cases against one fungus (*Candida albicans*- a yeast). Out of the 52 extracts screened for antimicrobial activity, 31(60%) showed some activity against one or more bacterial organisms used in the study (Table 4). In addition, seven out of 30 extracts tested displayed antifungal activity.

Table 4: Antimicrobial Activities of Selected Plant extracts

	Extracts and solvent		**Organisms**				
			Sa	Bs	Pa	Ec	Ca
1.	*Dorstenia multiradita* (whole plant)	CH_3OH	+	+	+	-	+
2.	*Dorstenia multiradiata* (whole plant)	CH_2Cl_2	+	+	+	+	+
3.	*Portulaca oleracea* (whole plant)	CH_2Cl_2	-	+	+	+	+
4.	*Portulaca oleracea* (whole plant)	CH_3OH	-	+	+	+	+
5.	Guava leaves (upper fraction)	H_2O	-	+	-	+	-
6.	Guava leaves (lower fraction)	H_2O	+	+	-	+	-
7.	*Momordica charantha* (aerial parts)	CH_2Cl_2	-	+	+	-	-
8.	*Combretum dulchipetalum* CH_3OH	fr. 10-30 (i)	+	-	+	+	-
9.	*Combretum dulchipetalum* fr.	40-50 (ii)	+	-	-	-	-
10.	*Combretum dulchipetalum* fraction	60-80 (iii)	+	-	-	-	-
11.	*Combretum dulchipetalum* fraction	80-100 (iv)	+	+	+	+	-

12.	*Dracaena manni* stem CH_3OH	fr.1-16 (i)	+	+	+	+	+
13.	*Dracaena manni* stem	fr.17-35 (ii)	+	+	+	+	+
14	*Dracaena manni* stem	fr.36-60 (iii)	+	+	+	+	+
15	*Eclipta prostrate* (aerial part)	CH_2Cl_2	+	-	+	-	-
16	*Scopora sp.* (whole plant)	CH_2Cl_2	+	-	-	-	-
17	*Brenania brieyi* (leaves)	CH_2Cl_2	+	-	-	-	-
18	*Erythrina senegalensis* (root)	CH_2Cl_2	+	+	+	-	-
19	*Morinda lucida* (leaves)	CH_2Cl_2	+	-	-	-	-
20	*Dorstenia multiradrata* (whole plant)	CH_2Cl_2	+	+	-	-	-
21	*Dorstenia multiradrata* (whole plant)	CH_3OH	+	-	-	-	-
22.	*Synedrella nodiflora* (whole plant)	CH_2Cl_2	-	-	-	-	-
23.	*Trema orientalis* (leaves)	CH_3OH	-	-	-	-	-
24.	*Phyalis augulata* (whole plant)	CH_3OH	-	-	-	-	-
25.	*Mitra carpus* (whole plant)	CH_2Cl_2	-	-	-	-	-
26.	*Morinda lucida* (leaves)	CH_2Cl_2	-	-	-	-	-
27.	*Crosophyta febrifuga* (roots)	H_2O	-	-	-	-	-
28.	*Pterocarpus angloensis* (leaves)	CH_2Cl_2	-	-	-	-	-
29	*Euphorbiaa hirta* (whole plant)	CH_2Cl_2	-	-	-	-	-
30.	*Sterculia tragacantha* (leaves)	CH_2Cl_2	-	-	-	-	-
31.	*Osbeckie sp* (aerial part)	CH_2Cl_2	-	+	-	-	
32.	*Synerdella nodiflora* (whole part)	CH_3OH	-	+	+	-	
33.	*Paropsia guineensis* (leaf)	CH_3OH	-	-	-	+	
34.	*Synclesic scabrida* (Aerial parts)	CH_3OH	-	+	-	-	
35.	*Paropsia guineensis* (stem)	CH_2Cl_2	+	-	-	-	
36	*Sterculea tragantha* (leaf)	CH_3OH	-	+	+	-	
37.	*Hoslundia opposta* (stem)	CH_3OH	+	+	-	-	
38.	*Ficus exasperata* (stem)	CH_2Cl_2	+	+	-	+	
39.	*Dracaena manni* (stem)	CH_2Cl_2	+	+	-	+	
40.	*Hoshinda opposita* (stem)	CH_2Cl_2	+	+	-	+	
41.	*Crassocephalum sp* (whole part)	CH_3OH	+	+	-	+	
42.	*Dorstenia multiradrata*(Whole part)	CH_3OH	+	+	-	+	

43	*Mussaenda elegans* (leaves)	CH_2Cl_2	+	+	-	+
44.	*Dorstenia multiradrata* (whole part)	CH_3OH	+	+	-	+

The bacteria used for screening these extracts were isolated from surgical wounds. They are resistant to Ciprofloxacin, Gentamicin and Ampicillin.

			E.c	B.s	Sal.	S.a	Shi
45.	*Ficus exasperata* (stem)	CH_2Cl_2	-	-	-	+	-
46.	*Dorstenia multiradrata* (whole part)	CH_3OH	-	-	-	-	-
47.	*Crassocephalum sp* (whole part)	CH_3OH	-	-	-	+	-
48.	*Dracaena manni* (stem) CH2Cl2	Fr.17-35	-	-	-	+	-
49.	*Dracaena manni* (stem) CH2Cl2	Fr.1-16	-	-	-	+	
50.	*Mussaenda elegans* (stem)	CH_3OH	-	-	-	-	
51.	*Morinda lucida* (leaves)	H_2O	-	-	-	-	
52.	*Voacanga africana* (leaf)	CH_3OH	-	-	-	-	

KEYS

+	=	Clear zone of inhibition
-	=	No zone of inhibition
B.s	=	*Bacillus subtilis*
S.a	=	*Staphylococcus aureus*
P.a	=	*Pseudomonas aerogenosa*
C.a	=	Candida albicans
CH_2Cl_2	=	Methylene Chloride
CH_3OH	=	Methanol

C. Anti- cancer and Anti- HIV Screening

As part of the plan to start the anti-HIV screening activities at InterCEDD, Nsukka, Dr. Barrows (University of Utah) visited Nsukka, Nigeria in December 2001. A detailed plan of action was worked out between Dr. Barrows and the participants in the project at Nsukka. Items of equipment required for the project were identified. One of the Nigerian participants (Dr. K. Chah) will visit Dr. Barrows' Lab at the University of Utah for hands-on training in anticancer and anti-HIV screening techniques.

A total of 21 samples of higher plants were processed and submitted to the group at Southern Research Institute for antiviral testing. The twenty-one samples were chosen based on ethnomedical uses.

1.1.3 Training Activities

A number of African scientists received training under the auspices of the ICBG during the period of this report. Training in phytochemistry took place at four sites: University of Mississippi (National Center for Natural Products Research), University of Dschang (Laboratory of Organic Chemistry, Department of Chemistry), University of Buea (Department of Chemistry) and International Center for Ethnomedicine and Drug Development Nsukka (InterCEDD). Scientists received stipends and reagents for carrying out research project under the auspices of the ICBG. Details of the individual programs are provided below.

University of Buea: Efforts at this center are largely devoted to the identification of centrally active agents. To this end, plants identified during the last report period as active in the CNS screen have been chosen for detailed phytochemical investigation. The following four students enrolled in the M. Sc. Program in Chemistry have been assigned to work on these plants:

- Erambo Ayokosok; Monoamine Reuptake Inhibitors of *Cassytha filiformis.*
- Edith Lepsia Fomunung: Neuroactive Constituents of *Triumfetta tomentosa.*
- Ruth Loh Viboh: Modulators of Monoamine Function in *Dracaena mannii.*
- Gregory Nkwe Nkepang; Monoamine Reuptake-Inhibiting Constituents of *Scoparia dulcis.*

An account of the follow-up work on these plants will be present in the next progress report.

University of Dschang: Efforts at this center have been directed at the isolation and structural characterization of plants secondary metabolites. Compounds are then submitted to the various centers for biological evaluation. Training in phytochemistry is undertaken at both the undergraduate and graduate levels. During the period covered by this report, the ICBG funded the training of 8 students at the graduate level. Out of this group, 2 students graduated with a Ph.D. in chemistry. Five students are currently enrolled in the Ph.D. program, and one of them has already passed the Ph.D. candidacy exam. The names of the students and the titles of their projects are provided hereunder.

- James Mbah: Title of Thesis: Antimalarial substances from Cameroonian medicinal plants: *Reneilmia cinucinnata, Glossocalyx brevipes* and *Vernonia guinensis.*
- Alembert Tchinda: Title of Ph.D. thesis: Contribution to the phytochemical study of some Cameroonian medicinal plants: *Vernonia guinenis* and *Noeboutonia glabresens.*
- Currently enrolled in the Ph.D. program:
- Christabel Tomla: Title of thesis project: Contribution to the phytochemical study of *Aframomum* species: *Aframomum sulcatum.*
- Michel Tchimene: Title of thesis project: Anti-malarial metabolites from Khaya species.
- Godfred Ayimele: Title of thesis: Contribution to the study of *Aframomum* species: *Aframomum sceptrum.*
- Sob Tanemossu: Title of thesis project: Contribution to the study of *Aframomum* species: *Aframomum letestuanum.*
- Virginie Ebessa: Title of thesis project: This is a new student.
- SimpliceTatsimo: Title of thesis project: Anti-malarial metabolites from Cameroonian medicinal plants: *Peninathus longifolius.*

Copies of the Ph.D. thesis will be submitted to the ICBG Program Office in due course.

INTERCEDD: ICBG is currently supporting two students (Chioma Ezeobi and Deborah Adazika) undergoing M.Sc training at the University of Nigeria, Nsukka.

University of Mississippi: Three graduate students enrolled in doctoral programs in Nigeria and Cameroon are working as Visiting Scholars at this site. The focus of their work is primarily to isolate and identify the active constituents of plants that were identified earlier in our bioassays as having anti-plasmodial and/or anti-protozoal activity. The names of the students and their projects and preliminary reports of their work are provided below.

- Julius Ngunde (University of Buea, Cameroon); Antiparasitic Constituents of Selected Cameroonian Medicinal Plants. Studies focus on *Peucedanum zenkeri, Glossocalyx brevipes, and Eriosema glomerata.*
- Christopher Ezugwu (University of Nsukka, Nigeria); Phytochemical Studies and Biological Screening of *Araliopsis tabouensis* (Rutaceae) and *Pachypodanthium staudtii* (Annonaceae).
- Odiri Onoruvwe (University of Jos, Nigeria); Isolation and Indentification of Antimicrobial and Antiprotozoal Constituents of *Chasmanthera dependens, Dorstenia, multiradiata and Enantia chlorantha.*

A progress report covering the period from January 2002 to June 2002 is presented below.

A. Topic: Phytochemical Studies and Biological Screening of *Glossocalyx Brevipes* and *Peucedanum Zenkeri*- Julius Ngunde

Introduction

Glossocalyx brevipes is a small tree, which grows in the equatorial forest of West and Central Africa. Our interest in *G. brevipes* was aroused by evidence of antileishmanial activity in preliminary bioassays. Its leaves are edible. *Peucedanum zenkeri* is a herb found in the West-Central African equatorial forest. Some cattle breeders use the leaf as a tobacco substitute. Members of this genus are widely used in Chinese folk medicine as expectorant, antitussive, antipyretic and stomachic. Coumarins isolated from *Peucedanum* genera possess some pharmacological properties. No work has yet been carried out on *Peucedanum zenkeri*. The reason to begin with *Peucedanum zenkeri* seed was its high activity observed in primary bioassays.

Aim

- Isolate and characterize the compounds present in those plants.
- Screen and evaluate the biological activities of the pure compounds.

Methodology:

Collection of Plant Material:

Glossocalyx brevipes was collected in November 2001 from the neighborhood of the Korup Project headquarters near the river banks of a secondary forest in Mundemba, Ndian Division in the South West Province of Cameroon, by Dr Chuyong George an ICBG Botanist. Dr. Claire Wirmum, Director of Medicinal Foods and Plants Bamenda, and an ethnobotanist of the ICBG

Project collected *Peucedanum zenkeri* in November 2001 in the forest of Babanki village of the North West Province of Cameroon.

Extraction

20 g samples of air-dried and ground plant material of the leaf and stem bark of *G. brevipes* were extracted with MeOH. The crude extracts were partitioned with Hexane, EtOAc, $CHCl_3$ and MeOH respectively. 20 g samples of air-dried and ground material of the seed, leaf and stem of both the cultivated and wild *P. zenkeri* were extracted with MeOH. All fifteen samples were submitted for biological screening. The extraction process continued with the bulk extraction of the leaf and stem bark of *G. brevipes* and the cultivated seed of *P. zenkeri*.

Results

Primary Biological Screening.

The following extracts showed activity from the results of the primary biological screening (antimalarial and antiprotozoal):

G. brevipes leaf: Hexane and EtOAc fractions

G. brevipes stem bark: Hexane and EtOAc fractions

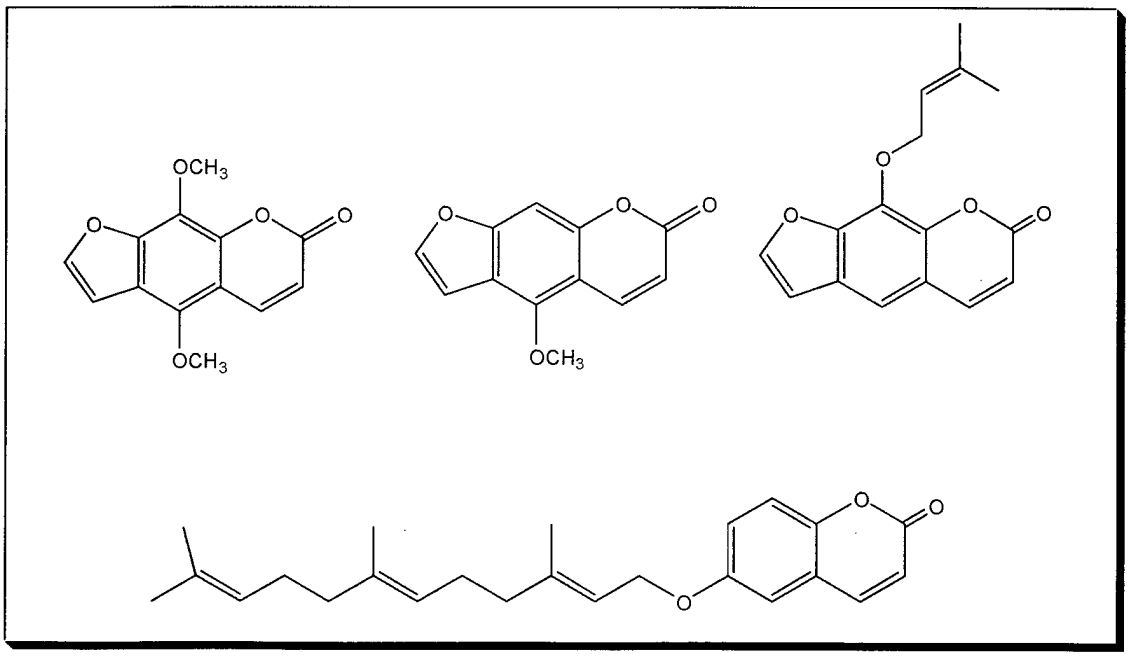
P. zenkeri cultivated: seed extract (generally 100 % activity)

P. zenkeri wild: seed extract (generally 100 % activity)

Isolation of the Major Constituents of the Seed of *P. zenkeri*.

The Hexane fraction was chromatographed on a column packed with silica gel. Now in its final phase, the column has so far yielded four coumarins: Umbelliprenin, Imperatorin, Bergapten and Isopimpinellin, and some fatty acids. All four have some documented pharmacological properties. Up to 2.0 g of imperatorin was isolated, and a similar quantity is expected from the $CHCl_3$ fraction. All compounds isolated are re-submitted for activity testing.

Figure 6 Structures of the compounds isolated from *P. zenkeri*



B. Phytochemical studies and Biological screening of *Araliopsis tabouensis* (Rutaceae) and Pachypodanthium staudtii (Annonaceae) - Ezugwu Christopher

Introduction

Araliopsis tabouensis Aubrev. Et Pellegr (Rutaceae) and *Pachypodanthium staudtii* Engl. & Diels (Annonaceae) are trees, which grow in the tropical forest of West and Central Africa. Various parts are used to remedy many ailments in African folk medicines. Previous work shows that the plants are rich in quinoline alkaloids and other secondary metabolites having several biological activities.

Aim

- To isolate, and determine the structures of the compounds present in the plant;
- Screen and evaluate the biological activities of the pure compounds.

Methodology:

Collection of Plant Material:

The plants were collected from Owai, Cross River State, Nigeria on the 6th of December 2001 and identified by Mr. Ozioko, a taxonomist at the Bioresources Development and Conservation Program at Nsukka. Voucher specimens are deposited at the B.D.C.P Herbarium.

Preparation of the plant material:

2 kg stem bark of *A. tabouensis*, 350 g stem bark and 930 g stem of *P. staudtii* were extracted at room temperature with methanol (yield: 340 g, 16 g and 30 g, respectively). 60 g of the methanolic extract of *A. tabouensis* was suspended in 1.0 N HCl (1L) and then ammonia was added to solution and partitioned with chloroform to obtain crude alkaloid extract. The alkaloid extract (8.0 g) was chromatographed on silica and eluted with increasing amount of MeOH in $CHCl_3$. Identical fractions were pooled and further purification was done at small scale using centrifugal chromatography (Chromatotron), gel filtration (Sephadex LH-20) and preparative TLC (Silica gel).

Results

Biological Screening

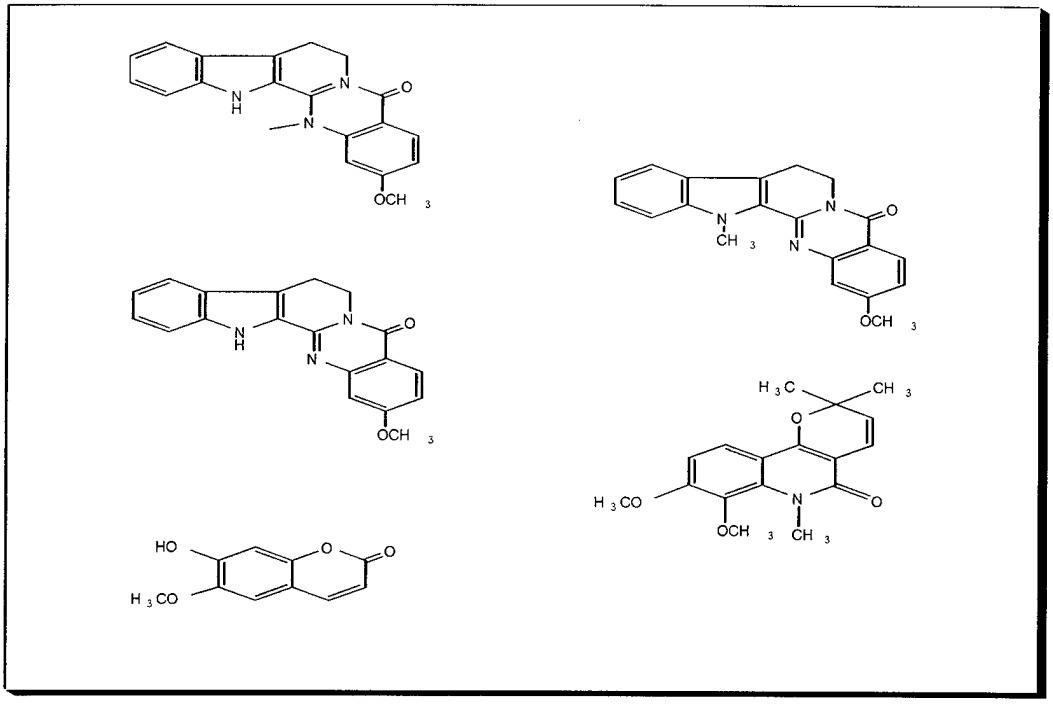
Hexane extract of *A. tabouensis* showed activity against *C. albicans* and *C. neoformans* while both EtOAc and Hexane extract of *A. tabouensis* showed activity against *Plasmodium falciparium.*

Phytochemical studies

Six compounds were isolated from *A. tabuoensis* and re-submitted for biological testing. The structures of the 5 compounds have so far been elucidated: 3 indolequinazoline alkaloids, a quinolone alkaloid and a coumarin shown below. We are in the process of isolating other constituents of *A. tabuoensis.*

Structures of the compounds:

Figure 7 Structures of the compounds isolated from *A. tabuoensis*



C. Isolation and Identification Of Antimicrobial and Antiprotozoal Constituents Of *Chasmathera dependens*, *Dorstenia multiradiata* and *Enantia chlorantha*. - Odiri Onoruvwe

The current project aims at isolating and identifying the anti microbial and anti protozoal constituents in three selected medicinal plants vis: *Chasmanthera dependens* Hochst. (Menispermaceae), *Dorstenia multiradiata* (Moracea) and *Enantia chlorantha* Oliv. (Annonaceae). In Nigeria, each of these plants is used for the treatment of different disease conditions, some of which suggest possible antimicrobial activities. *E. chlorantha* is widely used to treat malaria fever. (Oliver, 1960; Irvine, 1961; Ajali, 2002). The preliminary investigation involved solvent-solvent fractionation of the cold methanol extract of each plant sample (20-25 g) to yield hexanes, chloroform, ethyl acetate and water fractions. The extracts and fractions were sent for *in vitro* bioassay screening. Larger scale extraction of each plant part in a gradient fashion using solvents of different polarities, and chromatographic analyses of active extracts/fractions are currently being carried out. Resultant column fractions have been sent for further bioassay to test for anti microbial and anti malarial activities.

The *in-vitro* bioassay screening showed that the methanol extracts and, chloroform and ethyl acetate fractions of *D. multiradiata* and *E. chlorantha* have significant antimalarial antimicrobial activities. Some of the significantly active extracts and fractions showed no cytotoxic effects, while others were not significantly toxic. The result of the anti-leishmanial bioassay testing is being awaited.

Further studies will focus on the isolation of the active constituents from the column fractions as well as non-active chemical compounds. Using spectroscopic analysis and other methods, structural elucidation of isolated compounds for their identification will be carried out. The data generated from the present study will contribute to further information on these plants for ethnomedicine applications and databank for the shorter-term development of phyto-medicines and the more regular conventional medications.

2. ANTIMALARIAL DRUG DEVELOPMENT

In continuation of work previously carried out, we aimed to conduct chemical optimization of a number of natural product based ICBG compounds with promising antimalarial activities, whilst improving their pharmacokinetic properties. These include certain active constituents of *Cryptolepis sanguinolenta* and selected protoberberine alkaloids. Preparative scale isolation or the synthesis of already identified compounds for *in vivo* studies will also be undertaken. In addition chromatrographic methods will be developed for the separation and isolation of the active constituents of *Picralima nitida* and related indole alkaloids.

This report also describes a series of *in vitro* studies performed as part of the AP-3 Antimalarial Drug Development effort. Studies were conducted with purified fractions and isolated pure compounds. The procedure used measures the ability of the extracts/fractions/compounds to inhibit the incorporation of [G-^{3}H] hypoxanthine into the malaria parasites. Details of the protocol are described by Desjardins *et al* 1979 and Milhous *et al* 1985. Similar tests were performed using chloroquine and mefloquine as control standard drugs. We also examined the ability of extracts/fractions/compounds from our data bank to inhibit *Plasmodium falciparum* MRK and PK5 kinases. The results indicate that two compounds out of the 24 samples screened inhibited the enzyme MRK at 2.5ng/ml representing novel chemotype of compounds that preferentially inhibit Cyclin-Dependent Protein Kinases (CDKs).

2.1 Chemical Optimization of ICBG Lead Compounds with Antimalarial Potential.

A. Separation and isolation of the active constituents of Picralima nitida.

Using a combination of column chromatography (with silica) and preparative thin layer chromatography (silica gel plates) techniques, compound isolation from seed, stem bark and pericap (polar and non-polar) extracts have been performed. Over 20 constituents have been separated and are being analysed using mass spectrometry. Once they have been identified, any new constituent will undergo *in vivo* animal studies for activity and further investigation.
This has been a particularly laborious task considering each extract contains several (>8) constituents. However many of these are the same and are likely to be well characterized. The polar extracts were especially difficult to separate and purify.

Picralima nitida compound isolation from seed extract
Column chromatography: on silica, starting with 60% MeOH: 40% ACN with a gradual increase in polarity. Isolated 9 fractions, 3 contained more than one compound and underwent further isolation with preparative TLC to produce 5 additional compounds.

Picralima nitida compound isolation from stem bark (CH_2Cl_2 fraction)
Column chromatography: on silica, starting with 40% MeOH: 60% CH_2Cl_2 with a gradual increase in polarity. Isolated 1 compound (however, an extremely polar substituent could not be isolated).

Picralima nitida compound isolation from pericap (CH_2Cl_2 fraction)
Column chromatography: on silica, starting with 60% MeOH: 40% CH_2Cl_2 with a gradual increase in polarity. Isolated 3 fractions, 1 contained more than one compound and underwent further isolation with preparative TLC resulting in 3 additional compounds.

Picralima nitida compound isolation from pericap (MeOH fraction)
Column chromatography: on silica, starting with 60% MeOH: 40% CH_2Cl_2 with a gradual increase in polarity (extract contained a waxy insoluble substance which was not placed on column). Isolated 4 fractions, 1 contained more than one compound and underwent further isolation with preparative TLC to afford 3 additional compounds.

B. Synthesis of cryptolepine analogues.

Cryptolepine (Fig. 8**(1)**) isolated from *Cryptolepis sanguinolenta*, has long been employed as an antimalarial agent in traditional West African medicine. Several other alkaloids have also been characterized from this plant (Paulo *et al*, 1995). Its activity is 64.8 and 145.9 μg/ml against D6 and W2 strains respectively. The antimalarial activity is known of several cryptolepine analogues that have been synthesized (Bierer, 1997) and tested against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The three analogues with the greatest antimalarial activity (Fig. 8 (2-4)) have been used as a basis for further analogue design. It is planned that these compounds undergo further chemical optimization in order to enhance antimalarial and oral activity.

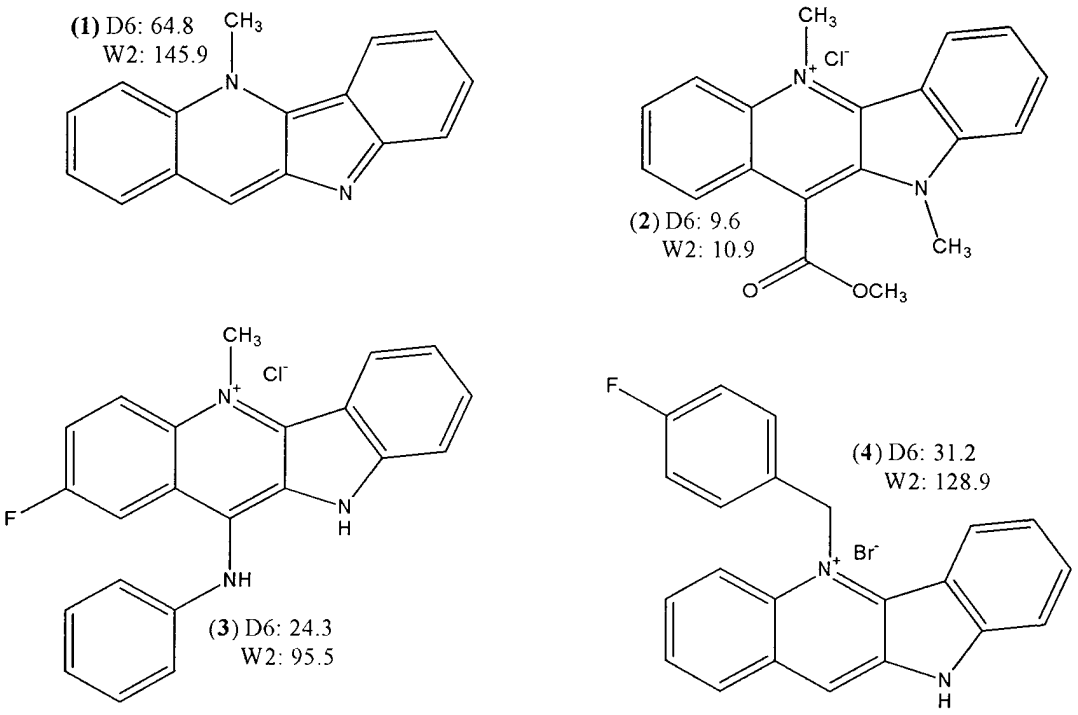


Fig.8: Structures of cryptolepine and previously synthesized analogues.

Two main synthetic routes have been employed for the preparation of further analogues. The first is based upon the Holt and Petrow (Holt *et al*, 1947) procedure (Fig. 9), which has been chosen due to its brevity and high yields. Other synthetic routes are longer and yields are significantly lower. Other analogues were prepared according to the route depicted in Fig. 10 (Bierer, 1997). Overall, these synthetic pathways have been time consuming but straightforward and minor problems have been overcome by modifications based upon other well-documented procedures (Bierer *et al*, 1998; Fan & Ablordeppy, 1997; Yang *et al*, 1999; De *et al*, 1997). To date, 10 analogues (Fig. 10) have been synthesized and a few more are planned. They are currently undergoing antimalarial and pharmacokinetic testing.

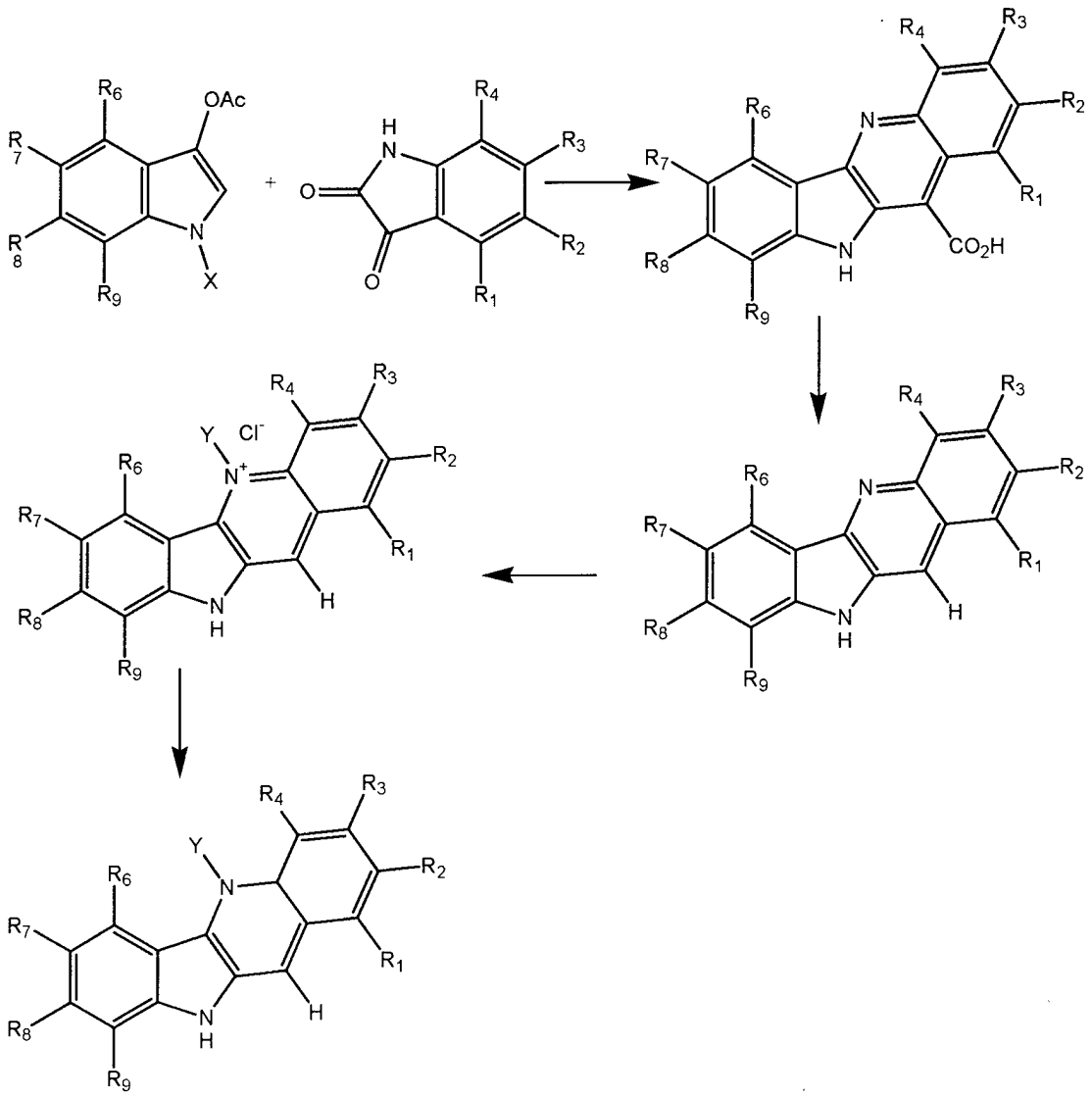


Fig.9: Scheme depicting synthetic pathway *via* the Holt & Petrow method.

C. *Synthetic Procedures:*

Quindoline-11-carboxylic acid

Isatin (4.25g, 28.6 mmol) in cooled aqueous KOH (25g in 115mL H_2O) was added under nitrogen, to indolyl acetate (5g, 28.6 mmol) with shaking. The mixture was left to stir vigorously for 3 days at room temperature. The reaction mixture was diluted with H_2O (65mL) and air was bubbled through while heating for 20mins between 75-80°C. The reaction mixture was filtered hot and the filtrate diluted with 200 mL ethanol. HCl (1M) was added until precipitate formed (~ pH4), which was subsequently filtered and washed with hot H_2O followed by ethanol. Dried orange powder (96.1%) in vacuum oven for 1 day, m.p: 321-326°C. ^{1}H NMR (DMSO-d_6) δ 11.45 (s, 1H, NH), 9.10 (d, 1H), 8.40 (d, 1H), 8.24 (d, 1H), 7.77 (d, 1H), 7.67 (m, 4H), 7.35 (t, 1H).

5,10-Dimethylquindoline-11-carboxylate

A mixture of Quindoline-11-carboxylic acid (4g, 13.5 mmol) in DMF (40mL), KOH (2.9g, 0.05 mol), BaO (8.1g, 0.05mol) and methyl iodide (11mL, 0.17mol) was stirred at room temperature for 48h. The reaction mixture was then partitioned between $CHCl_3$ (100mL) and H_2O (150mL). The organic layer was washed with H_2O (2x75mL), dried with Na_2SO_4 and evaporated. Recrystallization from hexanes afforded yellow crystals (85.5%), m.p: 122.5-123.5°C. ^{1}H NMR (DMSO-d_6) δ 8.40 (d, 1H), 8.25 (d, 1H), 8.00 (d, 1H), 7.70 (m, 1H), 7.38 (t, 1H), 4.20 (s, 3H), 3.81 (s, 3H).

5,10-Dimethyl-11-(methoxycarbonyl)quindolinium iodide

Stirred 5,10-Dimethylquindoline-11-carboxylate (0.6g, 2 mmol) in methyl iodide (5mL, 50 mmol) for 48h at room temperature. Evaporated excess methyl iodide in vacuo and recrystallized from methanol/diethyl ether to yield an orange solid (96.7%), m.p: 255.3-257.1°C. ^{1}H NMR (DMSO-d_6) δ 8.92 (dd, 2H), 8.35 (d, 1H), 8.24 (t, 1H), 8.08 (t, 2H), 8.04 (t, 1H), 7.68 (t, 1H), 5.10 (s, 3H), 4.33 (s, 3H), 4.02 (s, 3H).

5,10-Dimethyl-11-(ethoxycarbonyl)quindolinium iodide

10-Dimethylquindoline-11-carboxylate (0.3g, 1 mmol) was refluxed in EtOH (10mL) for 1.5h. Filtered resulting precipitate (89.7%), m.p: 243-245°C. ^{1}H NMR (DMSO-d_6) δ 9.08 (d, 2H), 8.55 (d, 1H), 8.34 (d, 1H), 8.08 (t, 1H), 7.99 (t, 2H), 7.68 (d, 1H), 7.49 (t, 1H), 4.85 (t, 2H), 4.06 (q, 3H).

5,10-Dimethyl-11-(butyloxycarbonyl)quindolinium iodide

10-Dimethylquindoline-11-carboxylate (0.3g, 1 mmol) was refluxed in BuOH (10mL) for 1.5h. Filtered resulting precipitate (72.6%), m.p: 238-239°C. ^{1}H NMR (DMSO-d_6) 9.04 (d, 1H), 8.35 (d, 1H), 8.28 (d, 1H), 8.07 (d, 1H), 7.77 (m, 4H), 7.42 (m, 1H), 4.15 (s, 3H), 3.78 (s, 3H), 3.60 (t, 2H), 1.41 (m, 2H), 1.25, (m, 2H), 0.95 (t, 3H).

5,10-Dimethyl-11-(benzyloxycarbonyl)quindolinium iodide

10-Dimethylquindoline-11-carboxylate (1g, 3.4 mmol) was refluxed in benzyl alcohol (30mL) for 2h. Allowed to cool and evaporated excess alcohol, recrystallized from acetone (%), m.p: 211-212°C. ^{1}H NMR (DMSO-d_6) δ 9.11 (d, 1H), 8.35 (d, 1H), 8.21 (d, 1H), 8.04 (d, 1H), 7.57 (d, 1H), 7.52 (d, 1H), 7.37-7.16 (m, 4H), 5.41 (s, 2H), 4.14(s, 3H), 3.99 (s, 3H).

11-Hydroxymethylquindolinium hydrochloride
5,10-Dimethyl-11-(methoxycarbonyl)quindolinium iodide (0.59g, 1.9 mmol) treated with $LiAlH_4$ in THF (1M) (10mL) under reflux for 45mins. Allowed to cool, then dissolve in EtOAc (~40mL). Filtered the precipitate and concentrated the filtrate. Column chromatography on silica (ACN:MeOH/ 70:30). Combined fractions, concentrated and acidified to produce an orange/yellow solid (36.7%), m.p: >280°C. ^{1}H NMR (DMSO-d_6) δ 9.60 (s, 1H), 8.90 (dd, 2H), 8.50 (d, 1H), 8.19 (t, 1H), 8.13 (m, 3H), 7.60 (t, 1H), 5.08 (s, 3H).

5,10-Dimethyl-11-(carbonyl chloride)quindoline
5,10-Dimethylquindoline-11-carboxylate (3.3g, 8.8 mmol) refluxed in $SOCl_2$ for 2h. Evaporated excess $SOCl_2$ *in vacuo* to give a bright red solid. Used without any further purification, m.p: 228-229.5°C. ^{1}H NMR (DMSO-d_6) δ 9.10 (d, 1H), 8.57 (d, 1H), 8.42 (d, 1H), 7.75 (m, 3H), 7.37 (t, 1H), 4.19 (s, 3H), 3.81 (s, 3H).

Quindoline
Quindoline-11-carboxylic acid (2g, 7.6 mmol) was refluxed in diphenyl ether (40mL) for 6h at 250°C allowed reaction mixture to cool then diluted with 25mL petroleum ether. Filtered mustard coloured precipitate (63.5%), m.p: 248-249.5°C. ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H, NH), 8.35 (d, 2H), 8.22 (d, 1H), 8.13 (d, 1H), 7.60 (m, 4H), 7.35 (t, 1H).

5-(4-Methyl)-benzylquindolinium hydrochloride
Quindoline (1.03g, 4.7 mmol), 4-methyl benzyl bromide (4mL, 16 mmol) and $CHCl_3$ (5mL) was left to stir in a sealed tube for 48h at 140°C. Dissolved reaction mixture in $CHCl_3$ (20mL) after cooling followed by ether (100mL). Filtered precipitate and washed with ether. Dissolved mustard coloured product in minimal $CHCl_3$ and purified with column chromatography; $CHCl_3$:MeOH (2%) on silica. Collected fractions and acidified (32.7%), m.p: 227.5-230°C. ^{1}H NMR (DMSO-d_6) δ 11.51 (s, 1H, NH), 9.10 (d, 2H), 8.92 (d, 1H), 8.75 (d, 1H), 8.54 (d, 1H), 8.48 (t, 2H), 8.28 (d, 1H), 7.81 (m, 8H), 7.40 (t, 2H), 7.13 (d, 2H), 7.06 (d, 1H), 5.83 (s, 3H).

5-(4-Nitro)-benzylquindolinium hydrochloride
Quindoline (1g, 4.6 mmol) and 4-nitro benzyl bromide (2.5g, 11.6 mmol) in $CHCl_3$ (5mL) was left to stir in a sealed tube for 48h at 140°C. Dissolved reaction mixture in minimal $CHCl_3$ (20mL) after cooling and diluted with ether (100mL). Filtered precipitate and washed with ether. Dissolved yellow coloured product in minimal $CHCl_3$ and purified with column chromatography; $CHCl_3$:MeOH (98:2) on silica. Collected fractions and acidified (22.3%), m.p: 243.5-245°C (decomp). ^{1}H NMR (DMSO-d_6) δ 13.08 (s, 1H, NH), 9.51 (s, 1H), 8.72 (d, 1H), 8.53 (d, 1H), 8.33 (m, 4H), 7.97 (m, 4H), 7.53 (t, 1H), 6.98 (s, 2H).

5-(2-Methyl)-propylquindolinium hydrochloride
Quindoline (0.5g, 2.3 mmol) and 1-bromo-2-methylpropane (2ml, 18 mmol) in DMF (3mL) was left to stir in a sealed tube for 48h at 140°C. Diluted reaction mixture with ether (100mL). Filtered precipitate and washed with ether. Dissolved dark orange product in minimal $CHCl_3$ and purified with column chromatography; $CHCl_3$:MeOH (98:2) on silica. Collected fractions and acidified (50%), m.p: 141-142°C. ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H, NH), 9.12 (d, 1H), 8.40 (d, 1H), 8.25 (d, 1H), 7.70 (d, 1H), 7.67 (m, 4H), 7.35 (t, 1H), 5.60 (t, 2H), 2.11 (d, 1H), 1.55 (m, 3H), 1.27 (m, 3H).

5-hexylquindolinium hydrochloride
Quindoline (0.5g, 2.3 mmol) and bromohexane (2mL, 14.3 mmol) in DMF (3mL) was left to stir in a sealed tube for 48h at 140°C. Diluted reaction mixture with ether (100mL). Filtered precipitate and washed with ether. Dissolved dark orange product in minimal $CHCl_3$ and purified with column chromatography; $CHCl_3$:MeOH (98:2) on silica. Collected fractions and acidified (42.3%), m.p: 129-130.5°C. ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H, NH), 8.97 (d, 1H), 8.40 (d, 1H), 8.29 (d, 1H), 7.70 (d, 1H), 7.67 (m, 4H), 7.36 (t, 1H), 4.55 (t, 2H), 2.08 (m, 2H), 1.91 (m, 4H), 1.64 (m, 2H), 1.17 (m, 3H).

2-Bromoacetamido-5-fluorobenzoic acid
2-amino-5-fluorobenzoic acid (2g, 12.8 mmol) in anhydrous DMF (5mL) and anhydrous dioxane (5mL) placed in a sealed flask and cooled to 0°C, bromoacetyl bromide (1.25mL, 13 mmol) was added slowly over 30mins (ensuring that temperature is maintained below 1°C). Left to stir overnight at room temperature. Added H_2O (40mL), filtered and washed the resulting white precipitate with H_2O (3x5mL) (93.2%), m.p: 196.2-196.9°C. ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H, NH), 8.17 (dd, 1H), 7.40 (dd, 1H), 7.13 (m, 1H), 4.00 (s, 2H).

5-Fluoro-2-[(N-phenylamino) acetamido]benzoic acid
2-Bromoacetamido-5-fluorobenzoic acid (5g, 18 mmol) and aniline (4mL, 44 mmol) in dry DMF (30mL) was refluxed at 120°C for 30h. After cooling, the reaction mixture was poured on to ice water (~200mL) and aq. KOH (5%) was added to adjust the pH to 10-11. Extracted with CH_2Cl_2 (3x100mL), then separated and acidified aq. layer with conc. HCl. The resulting white crystals were filtered (25.2%), m.p: 182-183°C. ^{1}H NMR (DMSO-d_6) δ 11.84 (s, 1H, NH), 8.73 (dd, 1H), 7.62 (dd, 1H), 7.45 (m, 1H), 7.11 (m, 2H), 6.65 (m, 3H), 3.91 (s, 2H).

2-Fluoro-11-quindolone
5-Fluoro-2-[(N-phenylamino) acetamido]benzoic acid (1.3g, 4.5 mmol) in PPA (45g) was heated at 130°C for 2h. Poured into crushed ice (~250mL), added saturated KOH to neutralize and extracted with EtOAc (2x300mL). Washed EtOAc fractions with water followed by brine. Dried and evaporated solvent. Purified via column chromatography using EtOH:MeOH (5:1) to produce a dark green product (70.4%), m.p: >300°C. ^{1}H NMR (DMSO-d_6) δ 12.65 (s, 1H, NH), 11.70 (s, 1H, NH), 8.20 (d, 1H), 8.05 (dd, 1H), 7.77 (dd, 1H), 7.60 (t, 1H), 7.53 (m, 2H), 7.21 (t, 1H).

2-Fluoro-11-chloroquindoline
2-Fluoro-11-quindolone (0.8g, 3.2 mmol) refluxed in $POCl_3$ (15mL) for 2h. Poured into ice water and neutralized with saturated KOH solution. Extracted with EtOAc (3x100mL), and washed the combined EtOAc fractions with water followed by brine, dried and concentrated solvent. Purification via column chromatography on silica, with EtOAc:Hexanes (1:6) produced a yellow solid (22.2%).

2-Bromoacetamido-6-chlorobenzoic acid
2-amino-6-chlorobenzoic acid (5g, 29 mmol) in anhydrous DMF (15mL) and anhydrous dioxane (15mL) placed in a sealed flask and cooled to 0°C, bromoacetyl bromide (4.4mL, 48.5 mmol) was added slowly over 20mins (ensuring that temperature is maintained below 1°C). Left to stir

overnight at room temperature. Diluted with cooled H_2O (100mL), filtered and washed the resulting white precipitate with H_2O (3x12mL) (97.4%), m.p: 125-127°C. ^1H NMR (DMSO-d_6) δ 10.08 (s, 1H, NH), 7.55 (d, 1H), 7.40 (t, 1H), 7.34 (d, 1H), 4.09 (s, 2H).

6-Chloro-2-[(N-phenylamino) acetamido]benzoic acid

2-Bromoacetamido-6-chlorobenzoic acid (10g, 17 mmol) and aniline (4.3mL, 46 mmol) in dry DMF (30mL) was refluxed at 100°C for 5h. After cooling to room temperature, the reaction mixture was poured on to ice water (~125mL) and aq. KOH (5%) was added to adjust the pH to 9. The resulting milky solution was extracted with CH_2Cl_2 (3x70mL), then separated and acidified (pH 3) aq. layer with conc. HCl and extracted with EtOAc (4x70mL). Combined the EtOAc washings, dried and evaporated. Recrystallized from EtOAc and hexanes (30.6%), m.p: 159-161°C. ^1H NMR (DMSO-d_6) δ 9.91(s, 1H, CO_2H), 7.95 (d, 1H), 7.41 (t, 1H), 7.28 (d, 1H), 7.12 (t, 2H), 6.57 (t, 3H), 3.85 (s, 2H).

1-Chloro-11-quindolone

6-Chloro-2-[(N-phenylamino) acetamido]benzoic acid (1.53g, 4.9 mmol) was heated with PPA (16g) at 120°C for 1h. Upon cooling to 60°C, it was stirred for 15min and treated with crushed ice. $NaHCO_3$ was added and the solid filtered. This was washed with hot water (200mL), cold water (50mL) and then dried in a vacuum oven. Dissolved the crude compound in minimal DMSO and diluted with H_2O. Filtered the resulting dark yellow precipitate and dried overnight in a vacuum oven (90.8%), m.p: >305°C. ^1H NMR (DMSO-d_6) δ 12.49 (s, 1H, NH), 11.61 (s, 1H, NH), 8.18 (d, 1H), 7.67 (d, 1H), 7.45 (m, 3H), 7.17 (m, 2H).

1-Chloro-11-chloroquindoline

1-Chloro-11-quindolone (1.2g, 4.5 mmol) was refluxed for 2h in $POCl_3$ (20mL). Allowed to cool to room temperature then poured into ice and neutralized with saturated KOH solution. Extracted with EtOAc (3x100mL), and washed the combined EtOAc fractions with water followed by brine, dried and evaporated solvent. Purification *via* column chromatography on silica, with EtOAc:Hexanes (1:6) produced a yellow solid (92.2%), m.p: 265°C (decomp). ^1H NMR (DMSO-d_6) δ 11.82 (s, 1H, NH), 8.38 (d, 1H), 8.20 (dd, 1H), 7.66 (m, 4H), 7.39 (dd, 1H).

1-Chloro-5-methyl-11-chloroquindolnium hydrochloride

1-Chloro-11-quindoline (1g, 3.5 mmol) in anhydrous toluene (60mL) was added to methyl triflate (1.5mL) and stirred at room temperature for 1 day. Filtered orange/yellow precipitate, then washed with ether (89.7%), m.p: 254-256°C (decomp). ^1H NMR (DMSO-d_6) δ 13.15 (s, 1H, NH), 8.82 (dd, 1H), 8.30 (s, 1H), 8.13 (d, 1H), 8.10 (d, 1H), 7.92 (d, 1H), 7.85 (d, 1H), 7.49 (d, 1H).

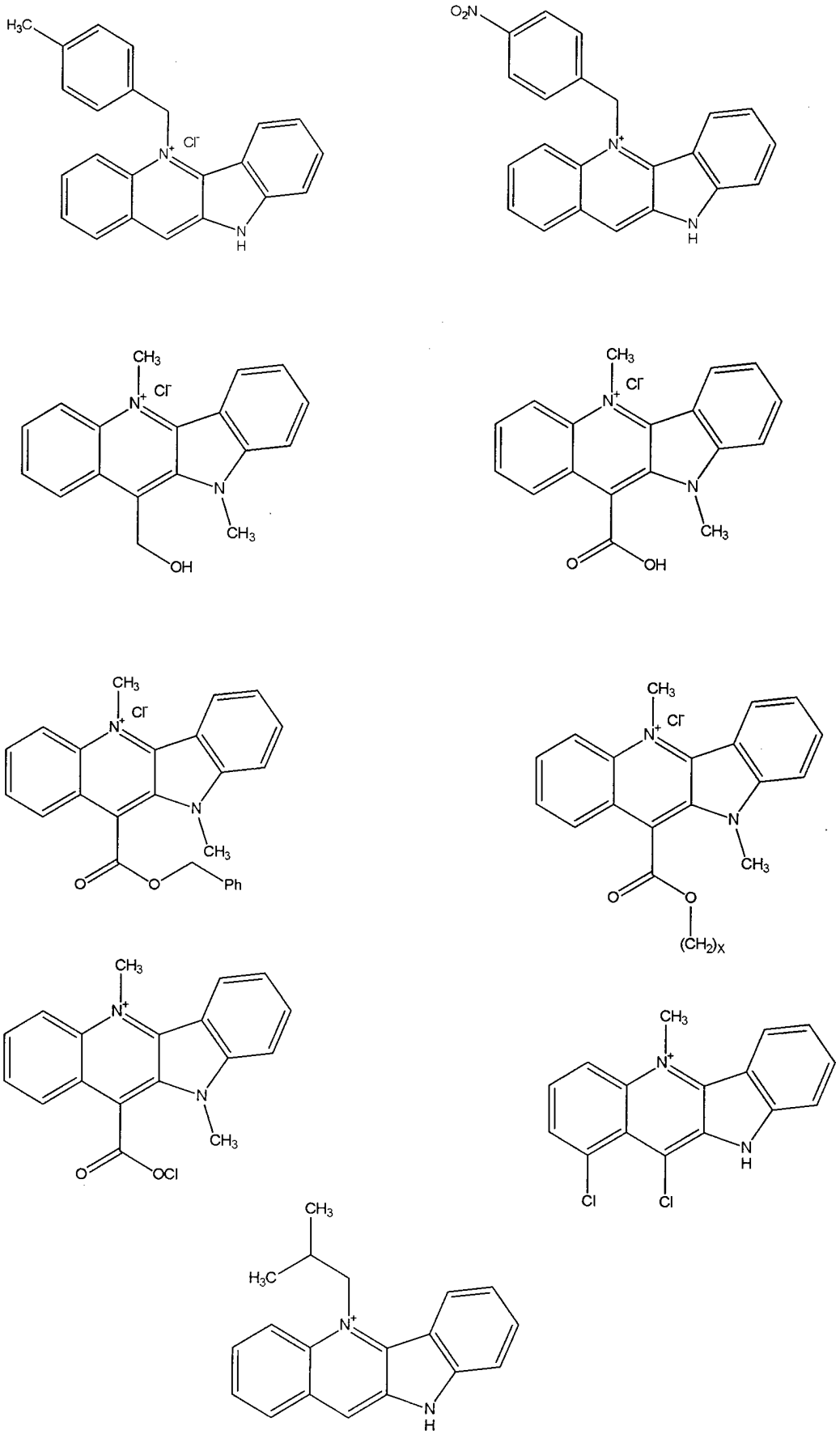


Fig.10: Structures of some of the analogues that have been synthesized.

The next generation of cryptolepine analogues takes pharmacokinetic parameters into greater consideration in order to achieve favorable ADME (absorption, distribution, metabolism and excretion). Thus, removal, addition, conversion of substituents and other structural modifications have been carried out. However, the same basic structure is maintained in order to avoid loss of activity. Analogue design will be facilitated if the pharmacophore of the lead compound can be identified. The pro-drug rationale is behind a number of those that have been synthesized and the use of bioisosteric replacement (thus maintaining electron density) is also employed. All analogues previously tested possessed the tetracyclic structure. Whether this is required for activity, is yet to be determined. In addition, on the basis of previous data, it appears that a completely delocalised structure is favored for activity. The planar aromatic structure is destroyed once the free base is formed. It is predicted that there will also be a reduction in activity. This also requires further investigation. One other route yet to be explored is the combination of cryptolepine with established antimalarials, which are becoming increasingly ineffective due to the emergence of resistance. This may prove to be a particularly successful route in the design of a cryptolepine analogue with antimalarial activity. With the identification of the pharmacophore that is essential for the activity of the cryptolepine analogues against drug-resistant *P. falciparum*, this class of compounds represents a new chemotype in the ongoing fight against malaria.

D. *Future work*

Upon completion of the preliminary antimalarial and pharmacokinetic testing, there will be continued optimization and characterization of identified lead compounds. In addition, the mode of action of these analogues is yet to be satisfactorily established. If it could be, this would aid the development of future analogues of this class of compounds. The spectroscopic characterization (UV, IR, MS, NMR, ^{13}C-NMR) of active constituents of *Cryptolepis sanguinolenta* will also be beneficial. In parallel, another approach yet to be undertaken is the synthesis of C-9 and C-3 alkyl derivatives of protoberberine alkaloids with improved pharmacokinetic properties. Finally, pilot scale isolation of indole alkaloids from *Picralima nitida* is planned for *in vivo* antimalarial bioassays.

2.1.2 Whole Cell Assays *(Plasmodium. falciparum)*

A total of 206 plants samples used in traditional medicine for the treatment of different forms of malaria were submitted for *in vitro* testing against *Plasmodium falciparum* at the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. So far, we have received results for 110 samples (Tables 6A-C) while the data for the remaining 96 samples are being expected. Separation and purification of the extracts of that revealed noteworthy effects in antimalarial bioassays and led to the isolation of a total of 22 compounds. The pure compounds were isolated mainly from the following five plant species: *Glossocalys brevipes*, *Penianthus longifolius* and *Homalium letestui*, *Picralima nitida* and *Khaya anthotheca*. A list of these samples and their *in vitro* IC_{50} values against two clones of *Plasmodium falciparum*, one sensitive to chloroquine (D6) and one chloroquine-resistant (W2) are shown in Table 6A-C. Extracts are designated Inactive (IC50 > 50, 000 ng/ml), Weak to Moderately Active (IC50 = 5,000 – 50,000 ng/ml) or Highly Active (IC50 < 5,000 ng/ml). Out of the 206 plant samples submitted for antimalarial screening (Table 6A-C), 19 plant species revealed noteworthy activity against D-6 or W-2 strains *Plasmodium falciparum*.

Table 5A: Summary of the Antiplasmodial Activity Extracts /Fractions/ Compounds showing ($IC_{50} \leq$ 3 µg/ml)

Plant Sample	Code	Lab No	Target		IC_{50} (ng/ml)
Control	Chloroquine		D6	=	2.656
Control	Chloroquine		W2	=	81.777
Euphorbia poinsonii	EPA	SU-2056	D6	=	2968.422
			W2	=	1542.585
Anogeissus leiocarpus	ALE3	SU-2061	D6	>	2500
			W2	=	2951.42
			W2	=	2162.125
Renealmia porypus	PRAA	SU-2081	D6	=	2899.03
			W2	=	1664.396
Penianthus longifolius	PLE	SU-2086	D6	=	350.066
			W2	=	284.377
Penianthus longifolium Stem bark	PL5	SU-2116	D6	=	24.4215
			W2	=	37.3638
Penianthus longifolium Stem bark	PL6	SU-2117	D6	=	67.4211
			W2	=	142.4276
Penianthus longifolium Stem bark	PL7	SU-2118	D6	=	26.6167
			W2	=	37.7054
Glossocalyx brevipes Leaves	GBM1	SU-2119	D6	=	702.5863
			W2	=	2125.7839
	GBM5	SU-2122	D6	=	1462.002
			W2	=	2552.9441
Glossocalyx brevipes	MTG3	SU-2124	D6	=	1326.2953
			W2	=	2373.7095
Glossocalyx brevipes	MTG4	SU-2125	D6	=	1164.9077
		SU-2125	W2	=	2367.5496
Aspilia africana (Aerial parts)	CH2CL2	SU-2175	D6	=	2145.6838
	CH2CL2	SU-2175	W2	=	2856.7156
Combretum dulchipetalum (Roots)	MeOH	SU-2178	D6		2000
		SU-2178	W2	=	1908.5365
Hymenocardia acida (Leaves)	CH2Cl2	SU-2153	D6	=	1949.9795
		SU-2153	W2	=	950.7654
Jatropha curcas (Leaves)	CH2Cl2	SU-2166	D6	=	2636.8037
		SU-2166	W2	=	1327.6487
Hyptis suaveolens (Leaves)	PET ETHER	SU-2158	D6	=	201.2735
		SU-2158	W2	=	158.0374

Table 5A contd. Plant Sample	Code	Lab No	Target		IC_{50}	IC_{9}
Guarea thompsonii (Stem Bark)	CH2Cl2	SU-2148	D6	=	2545.72	
		SU-2148	W2	=	777.8114	
Penianthus longifolius		SU-2194	D6	=	184.4256	
		SU-2194	W2	=	248.106	
Renealmia porypus		SU-2191	D6	=	2671.4805	
		SU-2191	W2	=	1261.1901	
Renealmia porypus		SU-2192	D6	=	1562.7423	
		SU-2192	W2	=	1156.9641	
Melian excelsa (Stem Bark)	CH2Cl2	SU-2151	D6	=	793.9665	
	CH2Cl2	SU-2151	W2	=	283.7151	
Hyptis senegalensis fr. 53-57	Leaves	SU-2252	D6		109.5586	245.9536
Hyptis senegalensis fr. 53-57	Leaves	SU-2252	W2		190.2197	321.3354
Hyptis senegalensis fr. 62-64	Leaves	SU-2254	W2	=	276.8698	433.6004
		SU-2254	D6	=	139.7729	437.1471
Hyptis senegalensis fr. 65-67	Leaves	SU-2255	D6	=	65.0873	169.262
		SU-2255	W2	=	118.1469	170.5544
Hyptis senegalensis fr. 68-69	Leaves	SU-2256	W2	=	145.6978	240.4132
		SU-2256	D6	=	119.3595	251.7279
Hyptis senegalensis fr. 70	Leaves	SU-2257	W2	=	427.5711	670.7832
		SU-2257	D6	=	307.6728	716.6232
Dicrocephala integrifolia (C)	Whole plant	SU-2292	W2	=	2978.639	3267.491
		SU-2292	D6	=	2935.179	3531.798
Eriosema glomerata (M)	Stem/Leaves	SU-2325	W2	=	1282.807	1656.092
		SU-2325	D6	=	1364.024	1777.928
Pittosprum viridiflorum (C)	Bark	SU-2336	W2	=	1397.6	2147.834
		SU-2336	D6	=	1035.728	3142.469
Pittosprum viridiflorum (M)	Bark	SU-2337	W2	=	2577.767	2913.585
		SU-2337	D6	=	2094.092	3124.385
Baillonella toxisperma (C)	Bark	SU-2343	W2	=	2850.672	3640.399
		SU-2343	D6	=	2430.021	7570.06
Triumfetta heudoletti (C)	"	SU-2352	D6	=	2824.606	3634.512
	"	SU-2352	W2	=	3011.981	3712.39
Aframonum pruinosum (C)		SU-2362	D6	=	2109.006	6710.301
		SU-2362	W2	=	2745.24	929489.8

The most active plant samples with IC_{50} less than 3.0 ug/ml against two strains of *Plasmodium falciparum* D6 and W2 are shown in Table 5A. The table indicates that nineteen plant species showed remarkable antiplasmodial activity. The most active compound was isolated from stem bark of *Penianthus longifolium* (MENISPERMACEAE) with IC_{50} values (D6= 24.42 and W2 =37.36) while the most active extract was obtained from the petroleum ether extract of the leaves of *Hyptis suaveolens* (LAMIACEAE) gave IC_{50} values (D6 = 201.27, W2 = 158.04). Bioassay-guided fractionation of the *H. suaveolens* extract also yielded fractions with lower IC_{50} when

compared with the extract. Other extracts with promising antplasmodial activity include *Eriosema glomerata, Pittosprum viridiflorum, Renealmia porypus, Melian excelsa and* ***Hymenocardia acida.***

Table 5B: List of Plants Displaying Moderate to High Antiplasmodial Activity

Plant Name	Plant Part/Solvent	Activity (ug/ml)-D6	Activity (ug/ml)-W2
Ancistrocladus barteri (Ancistrocladaceae)	Stem Bark (MeOH)	457.25	553.42
Enantia chlorantha (Annonaceae)	Stem Bark (MeOH)	133.93	121.65
Pachypodanthium staudtii (Annonaceae)	Unkown (MeOH)	126.75	138.60
Xylopia aethiopica (Annonaceae)	Seed (Hexane)	124.88	7955.10
Uapaca paludosa (Euphorbiaceae)	Bark (MeOH)	111.02	194.93
Amphimas pterocarpoides (Fabaceae)	Root (exudate)	1235.87	>5,000
Erythrophleum suaveolens	SK (pet ether)	>50.000	1820.83
Sida acuta (Malvaceae)	Leaf (Hexane)	143.828	13,978.12
Gnetum africanum (Gnetaceae)	Leaf (MeOH)	626.06	7093.16
Xymalos baillon (Monimiaceae)	Bark (CH2Cl2)	1846.90	3400
Glossocalyx brevipes (Monomiaceae)	Unknown (CH2Cl2)	762.27	2361.14
Xymalos baillon (Monimiaceae)	Bark (CH2Cl2)	1846.90	3400
Zanthoxylum bungei (Rutaceae)	Unknown (CH2Cl2-EtOH)	131.31	180.43
Eugenia uniflora (Myrsinaceae)	Leaf (MeOH)	2352.77	1220.77
Alchormea cordifolia (Euphorbiaceae)	Leaf (Hexane)	846.49	970.16
Fagara Lemairei (Rubiaceae)	Stem Bark (MeOH)	1697.82	1786.35

2.1.3 Molecular Target-Based Assays: Inhibitors of Plasmodial Cyclin-Dependent Protein Kinases (CDKs)

Effective inhibitors of *Plasmodium falciparum* CDKs can be identified by screening the ICBG plant database. This may lead to the discovery of a new class of anti-malarials. The CDKs project was based on previous work in the Department of Parasitology, WRAIR where these CDKs were characterized as potential antimalarial drug targets.

Background:

New drug discovery efforts in malaria today focus on target based drug screens to identify inhibitors of key enzymes vital to the survival of the organism. This study was undertaken to identify novel inhibitors of the malarial parasite that target a family of key enzymes.

Control of cellular growth and differentiation is highly regulated in all organisms in response to several intra- and extra-cellular signals. Cellular division ensures the propagation of the next generation of daughter cells that maintain the genetic integrity of the organism (Heuvel & Harlow, 1993). Cell growth is dependent on developmental cues as well as metabolic resources to ensure the proper timing of cell division. In some cases cellular transformation occurs when mutations

occur in the cell cycle regulatory mechanisms resulting in uncontrolled proliferation (Hall & Peters, 1996; Hunter & Pines, 1994). At the heart of the cell cycle machinery, Cyclin Dependent protein Kinases (CDKs) are the key regulators that ensure the cellular division occurs in a well ordered fashion (Heuvel *et al*, 1993).

CDKs are highly conserved in many eukaryotic organisms from yeast to man. In fact, yeast CDKs are able to restore normal cell cycle control in mammalian cells which contain mutations in several homologous CDKs (Lee & Nurse, 1987). This demonstrates that the CDKs not only share sequence homology but also they are functionally compatible among different species. The conservation of CDKs and their regulatory function implies that control of cellular division has evolved as a mechanism critical to the viability of the organism. In fact, this mechanism of cell division control relies heavily on the activity of the various CDKs (Grana & Reddy, 1995; Heuvel *et al*, 1993).

CDK activity is regulated by phosphorylation and association with regulatory proteins. Full activation of a CDK requires phosphorylation on a conserved threonine residue in a loop called the T loop and the association of a cyclin subunit (Morgan, 1995). Monomeric CDK lacks kinase activity due to several structural constraints. Without a cyclin subunit, substrates are denied access to the active site and key residues involved in the transfer of phosphate from ATP to the substrate are misaligned. The current model suggests that upon cyclin binding, the T loop moves away from the active site, allowing ATP to bind (Jeffery *et al*, 1995). Phosphorylation of the threonine residue within the T loop locks the CDK into its most active conformation. Additionally, conformation changes induced by cyclin binding orient the g-phosphate of ATP and facilitate the phosphotransfer reaction. Once active, the CDK phosphorylates proteins required for cell cycle progression. To ensure that cellular progression occurs in a sequential fashion, CDKs become inactivated by the targeted degradation of the cyclin subunit and the association of inhibitory proteins (Willems *et al*, 1996; Lee *et al*, 1987). In this type of sequential activation and deactivation, the cell cycle progresses so those critical events are completed before the cell commits to another round of proliferation.

In light of the CDKs role in cellular proliferation, they have become attractive drug targets for cancer therapy and the development of antifungal compounds (Meijer, 1996; Meijer *et al*, 1997; Saul & Battistutta, 1998). Several drug discovery efforts, which aim at either inhibiting the CDK directly or interfering with regulatory mechanisms required for CDK activation, have been reported. From these efforts, 6 classes of CDK inhibitors have been identified with one class of compounds now in clinical trials. Furthermore, inhibition of the cell cycle is widely considered as a new approach toward treatment for diseases caused by unregulated cell proliferation, including cancer (Schulze-Gahmen *et al*, 1996). Taken advantage of these developments and targeting Plasmodium CDKs may lead to the identification of a new class of anti-malarial compounds.

CDKs are highly conserved among eukaryotic species and, not surprisingly, several CDKs have been isolated from *Plasmodium* (Doerig *et al*, 1995; Ross-MacDonald *et al*, 1994; Vinkenoog *et al*, 1998). Unfortunately, the biochemical mechanism of regulation and the cellular role of these CDKs are not fully understood. A CDK from Plasmodium falciparum, known as Pfmrk, shares significant sequence homology with human CDK7 (46% identical, 62% similar) (Li *et al*, 1996; Waters *et al*, 2000). In mammalian cells, CDK7 and its cyclin H subunit function as the CDK

activating kinase (CAK), which phosphorylates the conserved threonine residue within the T loop of several CDKs (Fesquet *et al*, 1993). In addition to its role in CDK activation, CDK7 associates with the TFIIH transcription factor and regulates transcription and DNA repair (Serizawa *et al*, 1995). In this regard, CDK7 integrates cell cycle control with gene expression. It is possible that Pfmrk has a similar role in *Plasmodium falciparum* and this warrants further investigation of its regulatory and functional role. Homology with human CDK7 suggests that Pfmrk is in the position to regulate the other *Plasmodium* CDKs involved in cell growth and development.

Pfmrk functions at the extreme end of the cell signaling pathway, thus making it an attractive drug target. Based on homologous systems, inhibiting Pfmrk activity would shut down the cell cycle machinery resulting in the death of the parasite. This approach to antimalarial drug discovery is fairly new as it represents the first time that an approach has been undertaken to directly inhibit the machinery responsible for cell growth within the malaria parasite. CDK inhibitors currently under investigation include flavopiridol, olomoucine, roscovitine, puvalanol B, the dihydroindolo[3,2-d][1]benzazepinone kenpaullone, indirubin-3 -monoxime and novel diaminothiazoles such as AG12275. The anticancer therapeutic potential of CDK inhibitors has been demonstrated in preclinical studies, and Phases I and II clinical trials in cancer patients are currently underway (Buolamwini, 2000).

Therefore, screening ICBG plant database for identification and characterization of effective compounds that inhibit the *Plasmodium falciparum* CDKs may lead to the discovery of a new class of antimalarials.

Methodology:

Expression and Purification of *Plasmodium falciparum* CDKs was carried out by CPT Waters, Department of Parasitology, WRAIR (Waters *et al*, 2000; Buolamwini, 2000)

Kinase assays by autoradiography:

Purified Pfmrk (0.01 mg) was assayed in a 15ul kinase reaction containing kinase buffer (50 mM Tris-HCl pH 7.5, 10 mM MgCl2, and 1 mM DTT), 10 mg of Histone H1 (Upstate Biotechnology), 1 mg Pfcyc1, and 5 mCi [gamma-32P] ATP (Amersham). Reaction mixtures were incubated at 30 C for 15 minutes and stopped by the addition of SDS sample buffer, boiled, and resolved by 12% SDS-PAGE. Proteins were transferred to PVDF membrane, Coomassie stained, and exposed to film for autoradiography. PfPK5 and PfPK6 assays were performed in the same manner as above except that ribonucleotide reductase was used as substrate with PfPK6.

High throughput kinase assay:

The high throughput assay uses a 96 well microtiter plates that contains p81 phospho-cellulose filter paper in the bottom of each well to capture the phosphorylated substrate. These plates are used successfully in industry to assay for kinase inhibitors. This system includes Microtiter plates (Whatman Inc.), an automated sampler processor (Biomek Inc), microtiter plate shaking incubator, and a Top Count microtiter plate scintillation counter (Packard). The Biomek liquid handler has been programmed to pipet reagents required for the kinase reaction into each well of the microtiter plate. Total reaction volume per well in the working plate is 50 ml and includes kinase reaction buffer (50 mM Tris-HCl pH 7.5, 10 mM MgCl2, and 1 mM DTT), 10 mg of substrate, 1 mg

Pfcyc1, and 5 mCi [gamma-32P] ATP (Amersham). Prior to each assay, the Biomek serially dilutes drugs in a master drug plate and then transfers the diluted drugs to the working plates containing the components above. The last reagent added to the working plate is the [gamma-32P] ATP, which initiates the reaction. The working plate is then incubated for 30 minutes at 37 C. Following the incubation each well is washed 4 times with 250 ml of 1% phosphoric acid and then placed in the Top Count Scintallation counter to be measured for kinase activity. Each reaction is performed in triplicate to increase the accuracy of the assay. Several controls are included on each plate to include, background in the absence of kinase and kinase in the absence of drug to measure maximal activity. Based on these controls, the Top Count automatically computes the percent inhibition of each drug and reports it in a spreadsheet format.

The drug screen was conducted in two separate phases. Phase I consists of a pre-screen that measures inhibition of compounds to select those compounds demonstrating inhibition less than 50 mM. During phase II, selected compounds in phase I were evaluate to determine IC_{50} of those compounds. Such an approach eliminated the time and resources needed to evaluate compounds that do not make the minimum inhibition cut off. Compounds that were identified as effective inhibitor of a given Plasmodium CDK were evaluated against two other plasmodial CDKs.

A total of 24 ICBG samples consisting of extracts, purified fraction and compounds were screened against Plasmodium CDKs (MRK and PK5). The IC_{50} was recorded in ng/ml as shown in Table 6.

Results & Discussion:

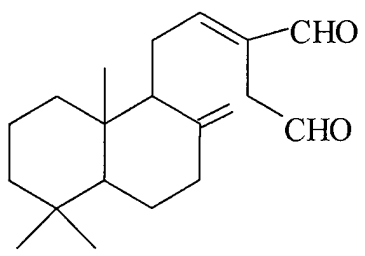
We examined the ability of these samples to inhibit *Plasmodium falciparum* MRK and PK5 kinases and found that the IC_{50} determined were in the low nanogram/ml range for 29% of the samples tested. Although both MRK and PK5 were inhibited to varying degrees, MRK is more susceptible to inhibition by our samples than PK5. As shown in Table 6, out of the 24 samples tested SU2243 (a diterpene isolated from *Aframomum daniellii* and SU-2258 (a hexahydroxyflavone) were among the first five lead compounds encountered in this assay. Both samples inhibited the enzyme MRK at 2.5ng/ml. Similarly the two samples also inhibited PK5 (IC_{50}, 40ng/ul). About 50% of the samples selected for this assay inhibited MRK. Although the nature of the inhibition remains unclear at this time, the results of this study represent the discovery of two compounds (SU2243 and SU-2258) that could serve as leads for new classes of CDK inhibitors.

Another class of compounds that significantly inhibited MRK and PK5 are the biflavonoids isolated from *Garcinia kola* seeds (Iwu, 1978; Okunji *et al.*, 2002). Working up the organic extract from 10.0g of *G. kola*, yielded four major compounds in sufficient amounts and purity (>98%). The compounds were identified by comparison of their spectral data and R_f values with those of the authentic isolates as 3", 3''', 4', 5,5'', 7,7''-heptahydroxy-4'''-methoxy-3,8"-biflavanone (Kolaflavanone); ",4',4''',5,5",7,7"-heptahydroxy-3,8"-biflavone (GB1); 7"-O-alpha-D-glucopyranosyloxy- 3",4',4''',5,5",7-hexahydroxy-3,8"-biflavanone. (GB1 glucoside) and 3",3''',4',4''',5,5",7,7'''-octahydroxy-3,8"-biflavanone (GB2). GB1 was the most active biflavanone (IC50, 5ng/ul) followed by kolaflavones and GB-2. The structures of these compounds are shown in Figure 11. In subsequent studies, we intend to compare the antiplasmodial activity of these

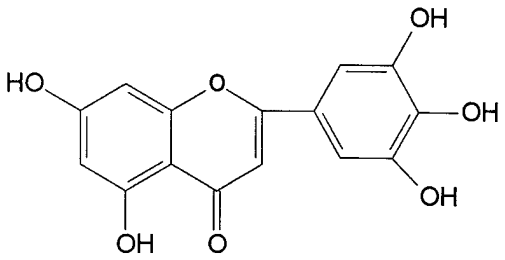
compounds with their activities in the CDK assay. Such a comparison may reveal a correlation between the two assays.

Table 6 Plant **Samples/Compounds Tested against MRK and PK5 Kinase Assay**

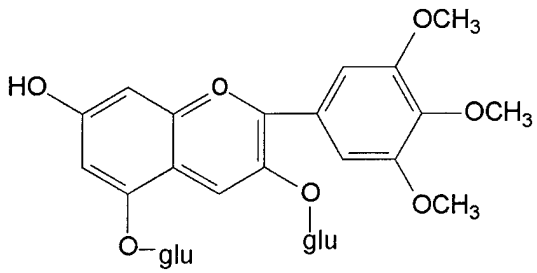
ID Number	Extracts/Compounds	MRK IC_{50} ng/ul	PK5 IC_{50} ng/ul
SU-2243	Labda-8(17),12-diene-15,16-dial	2.5	>40
SU-2244	Ajmalicine Hydrocholoride	>20	>40
SU-2245	Alstonine Tetrahydro	80	>40
SU-2246	Asculin	80	>40
SU-2247	BN 87724	>40	>40
SU-2248	BN 81553	40	40
SU-2249	Cinchonine	>80	>40
SU-2250	Colchicine	>80	>40
SU-2251	Curcumine	5	>40
SU-2252	Eserine Sulfate	>20	>40
SU-2253	*Dracaena manii* CMH(2:2:1)	>10	>40
SU-2254	*Garcinia Kola* LH-20 fr. 15	10	>40
SU-2255	*Garcinia Kola* LH-20 fr. 16	5	20
SU-2256	*Garcinia Kola* LH-20 fr. 20	10	>40
SU-2257	*Garcinia Kola* LH-20 fr. 23-25	20	40
SU-2258	Myricetin	<2.5	40
SU-2259	Lupeol	>10	>40
SU-2260	Malvin	>10	>40
SU-2261	Naringin	>40	>40
SU-2262	Palmatine Chloride	>40	>40
SU-2263	Morin	10	>40
SU-2264	Rauwolscine Hydrochloride	>40	>40
SU-2265	Solamargine	>40	>40
SU-2266	Ursolic Acid w/ HPLC	>10	40



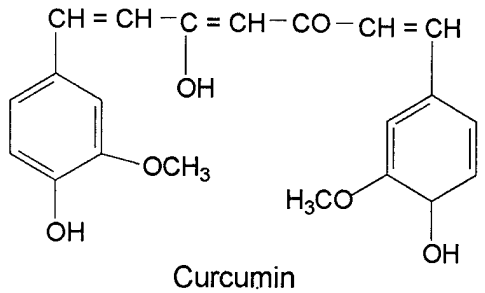
Labda-8(17),12-diene-15,16-dial



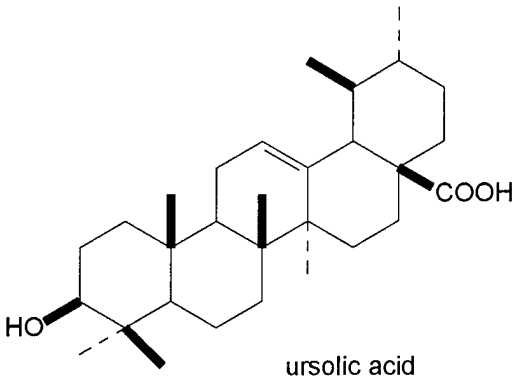
Myricetin



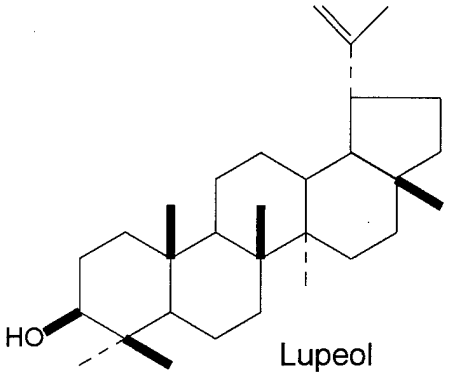
Malvin



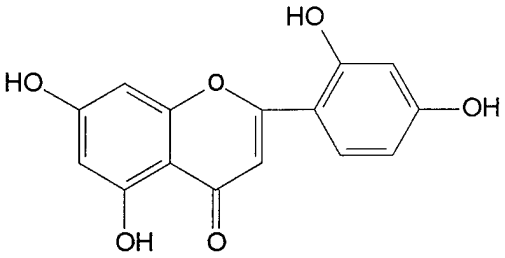
Curcumin



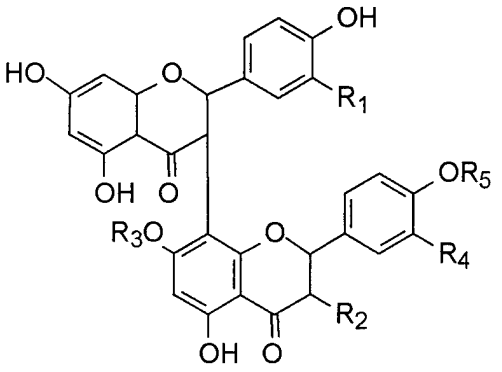
ursolic acid



Lupeol



Morin



	R_1	R_2	R_3	R_4	R_5
GB1	H	OH	H	H	H
GB1a-7"-O-glucoside	H	H	Glc	H	H
GB2	H	OH	H	OH	H
Kolaflavonone	H	OH	H	H	CH_3

Figure 11 Compounds Active against MRK Kinase at ≥ 5ng/ml

Table 6A

Antimalarial activity of plant extracts against *P. falciparum in vitro*

Plant Sample	Code	Lab No	WRAIR No	Target	ng/ml	<=>	IC_{50}
Control			Chloroquine	D6	1000	=	2.656
Control			Chloroquine	W2	1000	=	81.777
Homalium letestui	HL1	SU-2052	BP22782	D6	5000	>	5000
			BP22782	W2	5000	=	2564.11
Schefflera	Abo	SU-2053	BP22791	D6	5000	>	5000
			BP22791	W2	5000	>	5000
Khaya anthotheca	TKA	SU-2054	BP22808	D6	5000	>	5000
			BP22808	W2	5000	=	1468.094
Aframomum sulcatum	ADH	SU-2055	BP22817	D6	5000	>	5000
			BP22817	W2	5000	>	5000
Euphorbia poinsonii	EPA	SU-2056	BP22826	D6	5000	=	2968.422
			BP22826	W2	5000	=	1542.585
Euphorbia kinnii	EKA	SU-2057	BP22835	D6	5000	>	5000
			BP22835	W2	5000	=	2588.85
Euphorbia eutorrofilla	EEA	SU-2058	BP22844	D6	5000	>	5000
			BP22844	W2	5000	=	2034.394
Anogeissus leiocarpus	ALE1	SU-2059	BP22853	D6	5000	>	5000
			BP22853	W2	5000	>	5000
Anogeissus leiocarpus	ALE2	SU-2060	BP22862	D6	5000	>	5000
			BP22862	W2	5000	>	5000
Anogeissus leiocarpus	ALE3	SU-2061	BP22871	D6	5000	>	2500
			BP22871	W2	5000	=	2951.42
Lannea acida	LPA	SU-2062	BP22880	D6	5000	>	5000
			BP22880	W2	5000	>	5000
Inula klingii	IK	SU-2063	BP22899	D6	5000	>	5000
			BP22899	W2	5000	>	5000
Terminalia superba	TST	SU-2064	BP22906	D6	5000	>	5000
			BP22906	W2	5000	>	5000
Terminalia glaucecens	TGC1	SU-2065	BP22915	D6	5000	>	5000
			BP22915	W2	5000	=	2854.179
Terminalia glaucecens	TGC2	SU-2066	BP22924	D6	5000	>	5000
			BP22924	W2	5000	=	2620.585
Terminalia superba	TST2	SU-2067	BP22933	D6	5000	>	5000
			BP22933	W2	5000	>	5000
Aframomum sceptrum	ASS	SU-2068	BP22942	D6	5000	>	5000
			BP22942	W2	5000	>	5000
Garcinia kola	GSP	SU-2069	BP22951	D6	5000	>	5000
			BP22951	W2	5000	>	5000
Ellophobia spp	ELS1	SU-2070	BP22960	D6	5000	>	5000
			BP22960	W2	5000	>	5000

Table 6A contd

Plant sample	Code	Lab No	WRAIR No	Target	ng/ml		IC_{50}
Ellophobia spp	ELS2	SU-2071	BP22979	D6	5000	>	5000
			BP22979	W2	5000	>	5000
Anisopus mannii	ANIM	SU-2072	BP22988	D6	5000	>	5000
			BP22988	W2	5000	>	5000
Combretum glutinosum	CCG	SU-2073	BP22997	D6	5000	>	5000
			BP22997	W2	5000	>	5000
Combretum aculeatum	CCA	SU-2074	BP23001	D6	5000	>	5000
			BP23001	W2	5000	>	5000
Pteleopsis hylodendron	MPH1	SU-2075	BP23010	D6	5000	>	5000
			BP23010	W2	5000	>	5000
Pteleopsis hylodendron	MPH2	SU-2076	BP23029	D6	5000	>	5000
			BP23029	W2	5000	>	5000
Pteleopsis hylodendron	MPH3	SU-2077	BP23038	D6	5000	>	5000
			BP23038	W2	5000	>	5000
Pteleopsis hylodendron	MPH4	SU-2078	BP23047	D6	5000	>	5000
			BP23047	W2	5000	>	5000
Vitellaria paradoxa	PVA	SU-2079	BP23056	D6	5000	>	5000
			BP23056	W2	5000	>	5000
Renealmia porypus	PREA	SU-2080	BP23065	D6	5000	>	5000
			BP23065	W2	5000	=	2162.125
Renealmia porypus	PRAA	SU-2081	BP23074	D6	5000	=	2899.03
			BP23074	W2	5000	=	1664.396
Albizia ferruginea	AAF	SU-2082	BP23083	D6	5000	>	5000
			BP23083	W2	5000	>	5000
Marantochloa purpurea	MPA	SU-2083	BP23092	D6	5000	>	5000
			BP23092	W2	5000	>	5000
Marantochloa purpurea	MPAC	SU-2084	BP23109	D6	5000	>	5000
			BP23109	W2	5000	>	5000
Penianthus longifolius	PLA	SU-2085	BP23118	D6	5000	>	5000
			BP23118	W2	5000	>	5000
Penianthus longifolius	PLE	SU-2086	BP23127	D6	5000	=	350.066
			BP23127	W2	5000	=	284.377
Crotalaria incana	CIA	SU-2087	BP23136	D6	5000	>	5000
Control			Mefloquine	D6	250	=	7.827
			Mefloquine	W2	250	=	1.385

Table 6B Antimalarial activity of plant extracts/Fractions/Compounds against *P. falciparum*

				Synonym	Target	<=>	IC50
Penianthus longifolium	Stem bark	PL1	SU-2113	BP79030	D6	>	5000
				BP79030	W2	>	5000
Penianthus longifolium	Stem bark	PL3	SU-2114	BP79049	D6	>	5000
				BP79049	W2	>	5000
Penianthus longifolium	Stem bark	PL4	SU-2115	BP79058	D6	>	5000
				BP79058	W2	>	5000
Penianthus longifolium	Stem bark	PL5	SU-2116	BP79067	D6	=	24.4215
				BP79067	W2	=	37.3638
Penianthus longifolium	Stem bark	PL6	SU-2117	BP79076	D6	=	67.4211
				BP79076	W2	=	
142.4276							
Penianthus longifolium	Stem bark	PL7	SU-2118	BP79085	D6	=	26.6167
				BP79085	W2	=	37.7054
Glossocalyx brevipes	Leaves	GBM1	SU-2119	BP79094	D6	=	
702.5863							
				BP79094	W2	=	2125.7839
Glossocalyx brevipes	"	GBM2	SU-2120	BP79101	D6	>	5000
				BP79101	W2	>	5000
Glossocalyx brevipes	"	GBM3	SU-2121	BP79110	D6	>	5000
				BP79110	W2	>	5000
Glossocalyx brevipes	"	GBM5	SU-2122	BP79129	D6	=	
1462.002							
				BP79129	W2	=	2552.9441
Glossocalyx brevipes	Stembark	MTG2	SU-2123	BP79138	D6	>	5000
				BP79138	W2	>	5000
Glossocalyx brevipes	"	MTG3	SU-2124	BP79147	D6	=	1326.2953
				BP79147	W2	=	
2373.7095							
Glossocalyx brevipes	"	MTG4	SU-2125	BP79156	D6	=	
1164.9077							
				BP79156	W2	=	
2367.5496							
Uapaca paludosa	Stembark	Betulinic acid	SU-2126	BP79165	D6	>	5000
				BP79165	W2	>	5000
Khaya anthotheca		TKA1	SU-2127	BP79174	D6	>	5000
				BP79174	W2	>	5000
Khaya anthotheca		TKA2	SU-2128	BP79183	D6	>	5000
				BP79183	W2	>	5000
Khaya anthotheca		TKA4	SU-2129	BP79192	D6	>	5000
				BP79192	W2	>	5000
Khaya anthotheca		TKA6	SU-2130	BP79209	D6	>	5000
				BP79209	W2	>	5000

Control	MEFLOQUINE	D6	=	11.6708
Control	MEFLOQUINE	W2	=	4.7754
Control	CHLOROQUINE	D6	=	4.7697
Control	CHLOROQUINE	W2	=	
145.3013				

Table 6C Antimalarial activity of plant extracts against *P. falciparum in vitro*

PLANT NAME	**Plant Extract**	**Lab No. SU-NO**	***Plasmodium* Target**	**<=>**	**IC_{50} (ng)**
Asystasia gangetica	H2O	SU-2141	D6	>	12500
Asystasia gangetica	H2O	SU-2141	W2	>	12500
Asystasia gangetica	CH2CL2	SU-2155	D6	=	8406.8447
Asystasia gangetica	CH2CL2	SU-2155	W2	=	4802.1255
Pupalia lappacea	H2O	SU-2143	D6	>	12500
Pupalia lappacea	H2O	SU-2143	W2	=	7500
Lannea acida		SU-2186	D6	=	12502.6768
Lannea acida		SU-2186	W2	=	8313.3281
Uvaria chamae	CH2Cl2	SU-2146	D6	=	14644.3213
Uvaria chamae	CH2Cl2	SU-2146	W2	=	7454.4888
Uvaria chamae	MeOH	SU-2147	D6	=	8608.335
Uvaria chamae	MeOH	SU-2147	W2	=	4745.4888
Holarrhena floribunda (Leaves)	MeOH	SU-2164	D6	=	10775.6084
Holarrhena floribunda (Leaves)	MeOH	SU-2164	W2	=	5175.6792
Holarrhena floribunda (Leaves)	CH2Cl2	SU-2165	D6	=	8504.6807
Holarrhena floribunda (Leaves)	CH2Cl2	SU-2165	W2	=	3975.2646
Picralima nitida (Seed)	CHCl3	SU-2160	D6	>	12500
Picralima nitida (Seed)	CHCl3	SU-2160	W2	=	2163.3948
Picralima nitida (Seed)	HEXANE	SU-2161	D6	>	25000
Picralima nitida (Seed)	HEXANE	SU-2161	W2	=	7101.5361
Culcasia scanders (whole plant)	CH2CL2	SU-2157	D6	=	3816.0396
Culcasia scanders (whole plant)	CH2CL2	SU-2157	W2	=	1777.4214
Anisopus mannii		SU-2189	D6	>	25000
Anisopus mannii		SU-2189	W2	>	25000
Aspilia africana (Aerial parts)	CH2CL2	SU-2175	D6	=	2145.6838
Aspilia africana (Aerial parts)	CH2CL2	SU-2175	W2	=	2856.7156
Ritchiea capparoides (Roots)	MeOH	SU-2168	D6	>	25000
Ritchiea capparoides (Roots)	MeOH	SU-2168	W2	>	25000
Ritchiea capparoides (Roots)	CH2Cl2	SU-2169	D6	>	25000
Ritchiea capparoides (Roots)	CH2Cl2	SU-2169	W2	>	25000
Combretum dulchipetalum (Roots)	CH2CL2	SU-2176	D6		15000
Combretum dulchipetalum (Roots)	CH2CL2	SU-2176	W2	=	6021.9927
Combretum dulchipetalum (Roots)	H2O	SU-2177	D6	>	12500

Combretum dulchipetalum (Roots)	H2O	SU-2177	W2	=	6292.667
Combretum dulchipetalum (Roots)	MeOH	SU-2178	D6		2000
Combretum dulchipetalum (Roots)	MeOH	SU-2178	W2	=	1908.5365
Combretum dulchipetalum (Leaves)	H2O	SU-2179	D6		9000
Combretum dulchipetalum (Leaves)	H2O	SU-2179	W2	=	6084.103
Combretum dulchipetalum (Leaves)	CH2CL2	SU-2180	D6	=	5750.7998

Table 6C contd.

PLANT NAME	**Plant Extract**	**Lab No. SU-NO**	***Plasmodium* Target**	**<=>**	**IC_{50} (ng)**
Combretum dulchipetalum (Leaves)	CH2CL2	SU-2180	W2	=	2345.4836
Anogeissus leiocarpus		SU-2185	D6	=	9733.2979
Anogeissus leiocarpus		SU-2185	W2	=	7081.4946
Terminalia glaucecens		SU-2187	D6	=	3049.1604
Terminalia glaucecens		SU-2187	W2	=	2159.6104
Terminalia glaucecens		SU-2188	D6	=	3371.874
Terminalia glaucecens		SU-2188	W2	=	2986.4358
Combretum glutinosum		SU-2190	D6	=	5221.4712
Combretum glutinosum		SU-2190	W2	=	4761.0923
Hymenocardia acida (Leaves)	CH2Cl2	SU-2153	D6	=	1949.9795
Hymenocardia acida (Leaves)	CH2Cl2	SU-2153	W2	=	950.7654
Jatropha curcas (Leaves)	CH2Cl2	SU-2166	D6	=	2636.8037
Jatropha curcas (Leaves)	CH2Cl2	SU-2166	W2	=	1327.6487
Jatropha curcas (Stem)	CH2Cl2	SU-2167	D6	=	6687.3477
Jatropha curcas (Stem)	CH2Cl2	SU-2167	W2	=	3105.2563
Hymenocardia acida (Leaves)	MeOH	SU-2170	D6	>	12500
Hymenocardia acida (Leaves)	MeOH	SU-2170	W2	=	6405.7739
Hymenocardia acida (Leaves)	CH2Cl2	SU-2171	D6	=	3422.4402
Hymenocardia acida (Leaves)	CH2Cl2	SU-2171	W2	=	1326.8647
Phyllanthus amarus (Aerial parts)	CH2CL2	SU-2181	D6	=	4237.1372
Phyllanthus amarus (Aerial parts)	CH2CL2	SU-2181	W2	=	1916.8948
Euphorbia kinnii		SU-2183	D6	=	4591.0288
Euphorbia kinnii		SU-2183	W2	=	2168.1262
Euphorbia eutorrofilla		SU-2184	D6	=	10225.6299
Euphorbia eutorrofilla		SU-2184	W2	=	5375.6167
Cassia siamea	CH2Cl2	SU-2145	D6	=	7671.6602
Cassia siamea	CH2Cl2	SU-2145	W2	=	3608.8835
Homaltium letestui		SU-2182	D6	=	6517.5439
Homaltium letestui		SU-2182	W2	=	3507.427
Solenostemon monostachyus (Leaves)	CH2Cl2	SU-2152	D6	=	5584.895
Solenostemon monostachyus (Leaves)	CH2Cl2	SU-2152	W2	=	2381.5771
Hyptis suaveolens (Leaves)	PET ETHER	SU-2158	D6	=	201.2735
Hyptis suaveolens (Leaves)	PET ETHER	SU-2158	W2	=	158.0374
Hyptis suaveolens (Leaves)	CHCl3	SU-2159	D6	=	4999.1265
Hyptis suaveolens (Leaves)	CHCl3	SU-2159	W2	=	2727.4133

Solenostemon monostachyus (Leaves)	CH2Cl2	SU-2172	D6		8000
Solenostemon monostachyus (Leaves)	CH2Cl2	SU-2172	W2	=	4072.9399
Solenostemon monostachyus (W/P)	H2O	SU-2173	D6	>	12500
Solenostemon monostachyus (W/P)	H2O	SU-2173	W2	>	12500
Cassytha filiformis (Whole plant)	MeOH	SU-2162	D6	=	12723.3242
Cassytha filiformis (Whole plant)	MeOH	SU-2162	W2	=	9248.9561
Cassytha filiformis (Whole plant)	CH2Cl2	SU-2163	D6	=	6658.1289
Cassytha filiformis (Whole plant)	CH2Cl2	SU-2163	W2	=	4074.1946

Table 6C contd.

PLANT NAME	**Plant Extract**	**Lab No. SU-NO**	***Plasmodium* Target**	**<=>**	**IC_{50} (ng)**
Guarea thompsonii (Stem Bark)	CH2Cl2	SU-2148	D6	=	2545.72
Guarea thompsonii (Stem Bark)	CH2Cl2	SU-2148	W2	=	777.8114
Guarea thompsonii (Stem Bark)	MeOH	SU-2149	D6	=	11644.6729
Guarea thompsonii (Stem Bark)	MeOH	SU-2149	W2	=	8278.5352
Guarea thompsonii (Stem Bark)	H2O	SU-2150	D6	>	12500
Guarea thompsonii (Stem Bark)	H2O	SU-2150	W2	>	12500
Penianthus longifolius		SU-2193	D6	=	9503.3965
Penianthus longifolius		SU-2193	W2	=	12001.1133
Penianthus longifolius		SU-2194	D6	=	184.4256
Penianthus longifolius		SU-2194	W2	=	248.106
Prunus africana	CH2Cl2	SU-2174	D6	>	25000
Prunus africana	CH2Cl2	SU-2174	W2	=	10396.1895
Waltheria indica	MeOH	SU-2142	D6	=	3445.9602
Waltheria indica	MeOH	SU-2142	W2	=	3582.8086
Grewia cissoides	H2O	SU-2144	D6	=	3607.4185
Grewia cissoides	H2O	SU-2144	W2	=	2663.1753
Premna quadrifolia	CH2CL2	SU-2140	D6	=	9909.5068
Premna quadrifolia	CH2CL2	SU-2140	W2	=	5528.3281
Renealmia porypus		SU-2191	D6	=	2671.4805
Renealmia porypus		SU-2191	W2	=	1261.1901
Renealmia porypus		SU-2192	D6	=	1562.7423
Renealmia porypus		SU-2192	W2	=	1156.9641
Melian excelsa (Stem Bark)	CH2Cl2	SU-2151	D6	=	793.9665
Melian excelsa (Stem Bark)	CH2Cl2	SU-2151	W2	=	283.7151
Aspienium bulbiferum (whole plant)	CH2CL2	SU-2154	D6	=	13132.3945
Aspienium bulbiferum (whole plant)	CH2CL2	SU-2154	W2	=	8553.8164
Goyania long pelara (Leaves/Stems)	CH2CL2	SU-2156	D6	=	14360.4893
Goyania long pelara (Leaves/Stems)	CH2CL2	SU-2156	W2	=	7266.1099

2.4 Training:

Brian Harris, a high school student, interested in medicinal plants, worked at WRAIR utilizing ICBG/BDCP plant samples. The student learned techniques in extraction, chromatographic fractionation and isolation of bioactive compounds from African plants. The Science &

Engineering Apprentice Program (SEAP) summer program supported the student for a period of 8 weeks. The SEAP summer program is operated in conjunction with Department of Defense (DOD) laboratories and the George Washington University. Its purpose is to give academically talented students, ranging from Middle School through College, a hands-on experience in a scientific laboratory under the guidance of a mentor. The program lasts 8 weeks (8 hours per day - 5 days per week). Students are required to work the entire 8-week period and to produce a poster and a paper to document their work. The program offers students a unique and positive experience in their fields of interest, thus encouraging them to pursue careers in science and engineering. BDCP provided all the necessary plant materials as well as mentoring in the area of natural products. In addition, Dr. Foluke Fakorede, a post-doc, joined us to conduct chemical optimization of protoberberine alkaloids. The work is aimed at improving the pharmacokinetic properties of selected antimalarial compounds as well as conducting preparative scale isolation or synthesis of already identified compounds for *in vivo* animal studies. We also hired a technician, Ogo Nwabukwu, to provide technical laboratory assistance on the isolation of preparative quantities of active compounds from medicinal plants used in our antimalarial studies and also to assist in the processing of natural product samples for shipment to extramural laboratories for additional bioassays.

3. PLANT COLLECTION, ETHNOBOTANICAL STUDIES, AND ECONOMIC VALUATION

During this project-reporting period (September, 2001-June, 2002), we expanded the ethnobotanical studies and socio-economic evaluation of plant species to new areas. With the support of McArthur Foundation, we are in the process of conducting socio-economic valuation of two plant species in two communities in the Niger Delta.

3.1 Ethnobotanical studies:

The analysis of our ethnobotanical data in Cameroon has been completed. The Nigerian ethnobotanical studies, which cover a more heterogeneous population than Cameroon, have been continued with an increased emphasis on the Niger Delta region.

3.2 Plant Collection and Processing:

The Herbarium unit at InterCEDD collected 35 various plant parts (see Table 7) based on their use in ethnomedicine and dried them for extraction. Each plant was identified and a voucher specimen was prepared and deposited in the herbarium. Twenty other plants are currently being processed.

Table 7: The plant parts collected by InterCEDD

	Plant	Parts	Family
1.	*Hymenodictyon pachyantha*	stem	Rubiaceae
2.	*Crescentia macrocarpus*	leaves	Bignomcaceae
3.	*Petersianthus macrocarpus*		Lecythidaceae
4.	*Afromomum melegueta*	fruits	Zingiberaceae
5.	*Ocimum gratissium*	leaves	Lamiaceae
6.	*Vernonia colorata*	leaves	Asteraceae
7.	*Vernonia colorata*	stem	Asteraceae
8.	*Protea madeiensis*	leaves	Proteaceae
9.	*Protea madeiensis*	stem	Proteaceae
10.	*Vitex simplicefolia*	leaves	Verbenaceae

11.	*Funtumia elastica*	leaves	Apocynaceae
12.	*Jatropha gossypiifolia*	leaves	Euphorbiaceae
13.	*Jatropha gossypiifolia*	stem	Euphorbiaceae
14.	*Pterygota macrocarpa*	leaves	Sterculiaceae
15	*Ocimum virdis*	leaves	Lamiaceae
16.	*Ficus umbrella*	leaves	Moraceae
17.	*Ficus umbrella*	stem	Moraceae
18	*Acanthospermum hispidium*	aerial parts	Asteraceaae
19.	*Haemanthus multiflorus*	aerial parts	Liliaceae
20	*Stemonocloleus micranthus*	stem bark	Caesalpmiaiaceae
21.	*Uvaria anglolensis*	leaves	Annonaceae
22.	*Uvaria anglolensis*	stem	Annonaceae
23.	*Schumanniophyton problematicum*	leaves	
24.	*Brenania brieyi*	leaves	
25.	*Cleistopholis patens*	leaves	
26.	*Borreria venticilata*	aerial parts	
27.	*Hannao klaineana*	stem bark	
28.	*Hannao klaineana*	leaves	
29.	*Berlinia grandifolia*	leaves	
30.	*Pennisetum purpureum*	whole plant	
31.	*Ehretia cymosa*	leaves	
32.	*Ehretia cymosa*	stem	
33.	*Corchorus olitorus*	leaves	
34.	*Corchorus ollitorus*	stem	
35.	*Momordica charantha*	aerial parts	

3.3 Socio-economic Value Assessment

A) Summary

Although a number of laws exist that seek to protect the environment or promote sustainable development in Nigeria, national efforts to implement these have been poorly coordinated between bodies executing the legislation. The result is that there is a significant gap between policy formulation and implementation leading to duplications and fragmented approach to environmental economics.

In recognition of this constraint, our approach is based on adaptive management, which provides a framework to systematically extrapolate from lessons in project implementation to the development or execution of new projects (Fig 12). Such a framework directly addresses the lack of synergy in environmental management in the country as well as facilitates the full engagement of all stakeholders in project implementation.

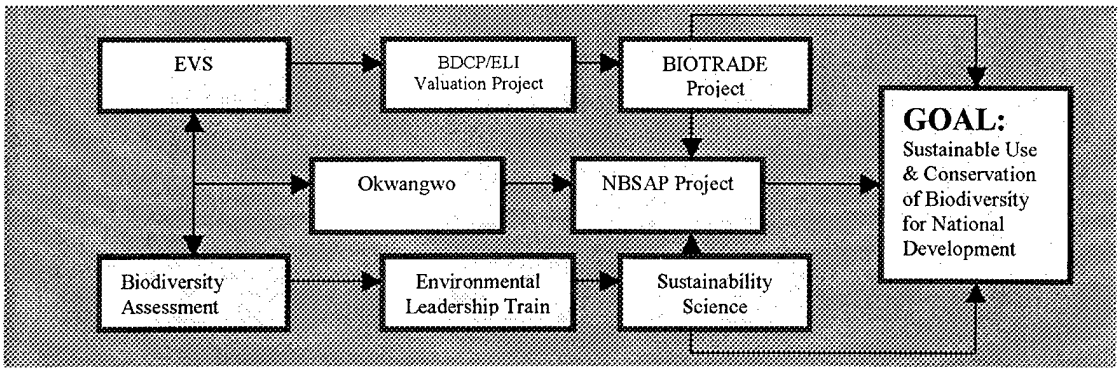


Fig 12: EVA framework

As the ICBG program ends its fourth year, the economic valuation program is establishing vertical and horizontal linkages, following a triple-helix model involving the scientific community, policy makers and the private sector (Fig 13). A fundamental aspect of this model is the recognition of the role of people and their local communities as the nexus of biodiversity use and management. This incremental approach expands the participatory base of biodiversity policy, sustainable use and management.

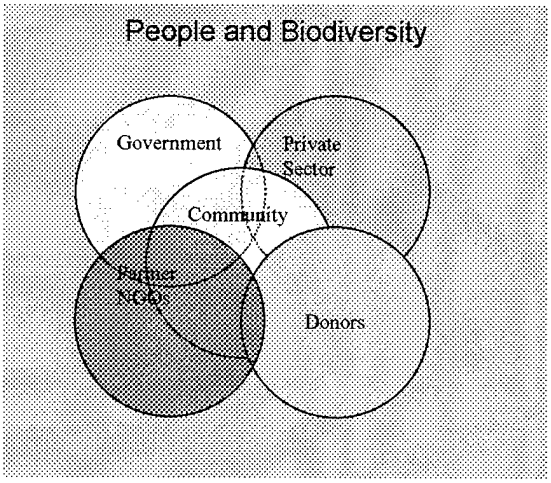


Fig 13: EVA Triple-helix model

B. Policy feedback:

i. Natural Resource Valuation Project:

Implementation of the economic value assessment program in Nigeria has established that imbalances in the law and practice of environmental valuation are central to the crisis the communities and ecosystems of the Niger Delta now face, as well as in the greater Nigerian environment. The indigenous value structure reflected by a reliance on NTFPs is not adequately captured by existing valuation and compensation laws in Nigeria. Recognition of the value of NTFPs could lead to their increased use as a tool in social and economic development, as well as to the development of a more complete understanding of the Nigerian environment. Additionally, more effective valuation practices could reduce conflict and civil strife due to inadequate compensation for damage wrought to the sources of food, water, and livelihoods of communities throughout the Niger Delta, as well as elsewhere in Nigeria.

Oil and gas extraction, development activities, and environmental accidents continue unabated in the Niger Delta; although the responsible decision-makers currently take into account only a small fraction of the full economic value of affected species and ecosystems. To respond to this growing

threat to both natural and human communities, the Bioresources Development and Conservation Programme (BDCP) and the Environmental Law Institute (ELI) seek to develop and advance approaches to ensure that natural resource decision-making incorporates the full economic value of natural ecosystems and their services. This work will contribute directly to the development of an integrated management plan for biodiversity conservation in the Niger Delta.

Specific Objectives

a.) The first objective is to establish the full economic value of one or two sample species in the Niger Delta that lie outside of official trade statistics and the gross national product (GNP), but at the same time play significant roles in the livelihood activities of local communities and their small producers. To accomplish this objective, partners will first establish, on the basis of existing models, an appropriate valuation methodology. They will then apply the methodology to selected species through the implementation of in-depth, on-the-ground case studies.

b.) The second objective is to develop and present recommendations for improving the legal framework governing natural resource valuation and natural resource damage assessment in Nigeria. These recommendations will be based upon a broad review of valuation literature, analysis of relevant legal frameworks in other countries, and interviews with legal specialists in the Niger Delta.

ACTIVITIES

To satisfy the project goal and objectives, the BDCP and ELI will work in close partnership to carry out two sets of integrated activities:

Valuing Sample Species

To meet the objective of establishing the full economic value of natural resources, two sample species in the Niger Delta will be evaluated. This work will be based upon the BDCP's own extensive experience and research in developing valuation methodologies, and also upon consultation with other professionals conducting valuation activities in West Africa and elsewhere.

Methodology

To collect information on wild resource use, researchers have generally combined a variety of conventional data gathering techniques, including questionnaire-based surveys, market surveys, time and motion studies, notebooks recorded by community members, etc. The most reliable of these methods require a large amount of time and resources, but yield fairly precise data sets for extensive statistical analysis. However in many instances less detailed information is required and/or less time is available; for example, in many project assessments or planning situations. In such cases, there is potential for making greater use of participatory assessment techniques, such as participatory rural appraisal (PRA). Emphasizing the importance of local people's understanding of their environment, participatory research methods can be very appropriate for addressing the often 'hidden' nature of wild resource use. The project seeks to develop a methodology, which uses a range of research approaches, including PRA and conventional social and biological science

techniques. The origins of PRA lie partly in the techniques devised to undertake farming systems research and this illustrates the conceptual link between the values that have been sought by PRA and the values, which are sought by conventional research techniques.

Household Survey/ Participatory Research Techniques

In terms of methodology, this study concentrated on the use of participatory research techniques. The aim of this section is to provide an introduction to the concepts of participatory research and highlight their relevance for valuing NTFPs (Table 8).

1. The Value of Indigenous Knowledge.
Participatory research has both a philosophical and practical interest in indigenous knowledge. Many people who use participatory research tools do so because they believe that research should be at least partly a participatory process, which involves, rather than exploits, the population being studied. In practice, this means that the views and opinions of local informants are paramount and should be understood. Researchers need to listen carefully to what informants have to say, and make an effort to understand the issues that confront them. (Schoonmaker-Freudenberger and Guèye, 1990)

2. Offsetting Bias.
Because PRA does not usually involve interviewing large samples of people, it is not possible to rely on numbers alone to guarantee a diversity of views. Special efforts have to be made to ensure that a range of opinions is represented and that all aspects of an issue are understood. In some situations, it is impossible to eliminate bias. Here participatory research acknowledges this and attempts to manage the effects of bias on the study. (Schoonmaker-Freudenberger and Guèye, 1990). This is achieved through explicit awareness of the context in which information is gathered (Pretty, 1993). Participatory researchers should be vigilant for sources of bias. Where bias exists, participatory research acknowledges this and seeks to understand the limits, which it imposes on research findings.

3. Triangulation.
Examining an issue from only one angle incorporates serious bias into the analysis. Therefore, participatory research looks at the same issue from different angles (Mettrick, 1993):

i) PRA uses a variety of information gathering techniques: key interviews, group discussions, secondary sources such as aerial photographs, transects, direct observation in addition to the participatory research tools.

ii) PRA uses a variety of units of observation: individuals, households, farms, and communities.

iii) PRA varies the composition of research teams so that their different perspectives can be brought to bear.

Table 8: Participatory Research Concepts

The use of participatory research techniques to assess the value of natural resources in the Niger Delta, and as a basis for reform of valuation standards and practice in Nigeria has both conceptual and practical advantages. Conceptually, the hidden nature of wild resources warrants an approach that emphasizes local-level knowledge and experience. Furthermore, certain participatory research tools, such as wealth ranking and seasonal calendars, are ideally suited to investigating the complex economic issues of `value to whom?' and when do they occur?' From a practical perspective, household and community-level values are a useful contribution to the community awareness aspect of the project.

Returns to Labor

Using information from a variety of sources, the returns to labor for harvesting and selling two NTFPs will be calculated. The returns to labor are simply the revenues less any relevant fixed or variable costs per unit of time devoted to the activity. These estimates provide a rough indication of the relative value of these resources since the return to labor captures both the value of the resource and the opportunity cost of labor. Where the opportunity cost of labor, and hence the actual economic value, are too difficult to assess, returns to labor are useful for understanding the economic importance of an activity only in the context of other available opportunities for people's time. A higher return to labor represents a greater incentive to engage in that activity, although opportunities may be limited due to social, cultural or other economic factors.

For all NTFPs, the groups of villagers harvesting them will be identified through a semi-structured interview with the village head and cross-checked with household heads and informants from the groups in question. The prevailing agricultural wage within villages is an interesting benchmark against which to compare the returns to labor. The wage paid for agricultural work will vary depending on the amount of effort required for the task and the abilities of the laborer.

Market Values

Information from a variety of sources, including previous studies, will be combined to assess the gross market value of non-timber forest products harvested.

Where possible, estimates will be obtained of the number of people involved in each activity. The market value is the total amount of resource harvested, whether marketed or not, valued using market prices from markets in the local communities to be sampled. The market value provides a rough indication of the importance of each resource to the local economy, in terms of its overall scale and in comparison to other activities. This calculation would be the first step in calculating the economic value. The estimated financial value of agricultural production will be provided for comparative purposes.

Estimates of the aggregate amount of harvesting for the various wild resources will be calculated using information about the number of people engaging in each activity. This will be estimated through a pile-of-stones discussion with a group of men and then crosschecked with village and ward heads, as well as individual informants involved in other interviews/discussions.

Changes in Resource Availability

A description of the values generated by resources at one point in time says little about their sustainability. It is helpful therefore, to ascertain whether any of the resources, particularly those with high values, appear to be declining in abundance, due to harvesting or other changes in their availability. In the survey, attempts will be made to determine the extent to which ecosystem changes affect the supply of economically important resources.

In this study, changes in the availability and harvesting of resources over time will be investigated primarily by means of historical matrices and the results will be more qualitative, involving relative changes. In the absence of archival records or other secondary sources, this is the only available means to assess initially the changing state of resources. Any indications of scarcity

could provide the basis for considering a more in-depth study, including the use of biological sampling techniques.

Sampling Plan

Based on a two-stage methodology, the team will consider a list of 10 species or products that include both NTFPs and a few agricultural products.

[i] Identification of species of local importance according to:

- Economic criteria
- Social/cultural criteria
- Spiritual value criteria

[ii] Then, the team will score each species based on the selection criteria, starting out with "cross-cutting" criteria:

- Importance of species / products to local communities and small producers;
- Potential to contribute to conservation and sustainable development (livelihoods) by mitigating threats to species; and
- Potential for generating alternative income, providing communities with improved livelihoods.

[iii] The next steps will include the following:

Further and more intensive marketing research on the two highest ranked species, resulting in "product packets" with comprehensive data on:

- Prices,
- Markets and companies,
- Research on local trade networks
- Socio-economic, ecological, and cultural features of product use and trade

Combination of [iii] with the above "product packets" will form a comprehensive informational and technical basis from which to make informed decisions. Comparative (and competitive analysis) will be conducted for the selected species analysis) will be conducted for the selected species / products and allow decisions based on relative benefits and costs.

The number of communities to be sampled will be limited to two, due to constraints in time and resources. The number of households to be sampled in the two communities will be one hundred. A semi-structured questionnaire will be used to conduct interviews with village heads. Other interviews will be conducted in groups, using PRA techniques.

ii. National Biodiversity Strategy and Action Plan (NBSAP) Project

Background:
Biodiversity is often misunderstood and in the minds of many, it is inexhaustible. Recent developments further worsen the case, as sustainable use is not practiced. The natural ecosystems

in Nigeria used to host a high degree of biodiversity which is poorly researched and largely undocumented. The exact number of wild plants and animal species occurring in the country including endemic species are not known. Yet the biotic species are constantly being used by rural communities to sustain their livelihoods. This use includes for food, medicine, culture and other auxiliary uses that are now being better recognized. Unfortunately, at the extreme, fiscal policies aimed at national development are instruments used to further deplete the nation's biodiversity through urbanization, road construction, oil extraction and other exploitation processes. Traditional farming systems and uncontrolled harvesting of biological resources have led to the loss of the biodiversity in the country. In the circumstances, it is becoming extremely difficult to adequately design programs for biodiversity conservation in the absence of realistic data as many taxa of flora are still not documented and some have been lost due to extreme misuse.

It was on the basis of the foregoing that Nigeria signed the convention on biological diversity (CBD) in 1992 during the Earth Summit, to among others design an international pathway for the conservation and utilization of biodiversity. A draft National Action Plan for biodiversity conservation was prepared earlier, but this seemingly desk report was found to be deficient in the inclusion of data generated through participatory rural appraisal, a process which brings people into the development process.

In order to implement Article 6 and 8 of the CBD, the Federal Ministry of Environment has engaged the services of six consultants to fill the gaps identified in the first version of the biodiversity strategy and action plan through participatory approach (PRA) at the grassroots, with BDCP as the lead civil society institution. The country was divided into four zones vis: North-East, North-West, South-East and South-West for data collection. The series of data generated was reviewed at workshops held at the zonal level. The zonal workshops were designed for stakeholders in each zone to participate in reviewing the reports of consultants in order to get a truly representative document for each zone. In turn, the zonal reports will form a working document for the first National Workshop, which is designed to collate the views of all stakeholders under a cross sectional approach.

WORKSHOP OBJECTIVES

The overall objective of the workshops was to involve stakeholders at the grassroots under a participatory approach to review as well as collect current data on the role and status of biodiversity in the country. The specific objectives were to:

i. Document the perceptions, aspirations, and the problems of biodiversity utilization and conservation at the community level;
ii. Examine the effects of traditional practices and how these can be improved upon to conserve biodiversity.
iii. Assess the role and uses of biodiversity, identify trends in land use, and determine indigenous conservation strategies and human condition index in the use of wild plants and wild animals in rural communities.
iv. Create a general awareness about the existing opportunities and gains that would accrue to communities under a collective support system for biodiversity conservation.

v. Use the data collected from the workshops to develop programs for efficient and effective conservation of biodiversity in the country.

WORKSHOP METHODOLOGY

I. The presentations at the zonal and national workshops were through in-house delivery of reports by Consultants.

These were followed by group discussions on issues raised. Participants asked questions while consultants provided answers. Audiovisual materials were used to facilitate participants' understanding of the main issues raised during the workshop.

II. Participation at the workshop involved the following stakeholders:

i. Representatives of communities from the three senatorial districts of participating States in each zone.
ii. Directors of Forestry, Agriculture, Public Health and Environment in each State or their representatives.
iii. Representative of a Research Institute or institutions of higher learning in the State and the academia in general.
iv. Representative of the private sector.
v. NGOs, CBOs and members.
vi. The Technical Committee of the project.
vii. The workshop consultants (the lead consultants, the national consultants).

III Participants at the workshops were constituted into three working groups to brainstorm and discuss some key issues related to the workshop. The topics discusses were earlier identified by the National Consultant. These include:

a) Sustainable utilization and incentive structure for biodiversity conservation.
b) Traditional conservation practices
c) Conservation issues in the zone

IV The workshop program in each zone and at the national level included opening ceremony and presentation of reports. Each presentation was followed by plenary discussions that examined and addressed critical issues in the report and vital biodiversity conservation issues.

After the discussions by the three working groups, they were collapsed to form two other groups on the workshop recommendations and resolutions respectively, at the Zonal levels.

This process, though hectic was valuable in reviewing and collecting more data to actually fill the gaps in the available document in the conservation status and needs of Nigeria's biodiversity.

iii. Nigeria Biotrade Program

The Biotrade program is an integrated approach towards trade biodiversity conservation and sustainable development, initiated by the United Nations Conference on Trade and Development (UNCTAD) in November 1996 to provide technical and financial support to enable developing countries to maximize the potential economic value of their biological resources.

The BIOTRADE Initiative seeks to enhance the capability of developing countries to produce value-added products and services from biodiversity for both domestic and international markets (Fig 14). It is an integrated program consisting of three complementary components: the BIOTRADE country program; market research and policy analysis; and Internet services.

Fig 14: The world market of products and services derived from biological resources is estimated to be more than US$900b per annum. The world market for herbal remedies in 1997 amounted to US$16.5b. The organic food and beverage market for Japan, the US and EU is approximately US$20b for 2000. The market for natural coloring and flavoring materials is estimated at US$150m worldwide. High demand exists for fresh and processed fruits and vegetables. Among developing countries, India captures 2.5% of the global market for herbal products. It is estimated that Cameroon exports US$150m yearly in medicinal plants.

Like many developing countries, Nigeria is endowed with rich and highly diverse biological resources. These resources provide a wide range of products and services, such as watershed protection, carbon sequestration, eco-tourism, and products derived from bioprospecting, intermediate products (e.g. natural dyes, colorants, oils, biochemicals compounds, medicinal extracts) and final products (e.g. timber, handicrafts, nuts, fruits, perfumes, medicines). Many of these products are collected for subsistence use. Some of them have served as an important source of innovation for the pharmaceutical, biotechnology, cosmetic or agrochemical industries.

The potential economic value of biodiversity should be translated into tangible economic benefits for populations whose livelihood depends on biodiversity. One of the ways to achieve this is by taking advantage of the new investment and trade opportunities that are emerging for biodiversity-based products and services. Interest for these products is on the rise because of the intensifying search of industries for recyclable products, the globally emerging biotechnology industry, and shifts in consumer behavior in industrialized countries and urban areas of developing countries.

It is our opinion that if Nigeria is able to seize these opportunities, biodiversity could be turned into an engine for growth and sustainable development.

To achieve this objective, we have proposed the initiation of a Biotrade-Nigeria program in collaboration with the Federal Ministry of Agriculture and Rural Development (FMARD) with UNCTAD and BDCP as technical partners.

Responding to the Challenge

BDCP/ICBG members recently represented Nigeria at the G-15 Meeting on "Trade and Investment in Biodiversity Products and Services, Intellectual Property Rights and Traditional Knowledge" in Caracas, Venezuela, 3-4 April 2002, where initial contact was made with UNCTAD representatives.

Establishing a BIOTRADE program in Nigeria requires a focal point in government with the requisite mandate for food and related products. The FMARD best meets the institutional capacity and capability for this program. Accordingly, the BDCP is currently exploring strategies for implementing this initiative with FMARD by examining the following:

- Technical requirements for initiating a Biotrade program;

- Legal and policy frameworks;
- Identification of all relevant stakeholders;
- Cooperation agreements (including initiation of MOU)
- Timeline and modalities for implementation.

The BIOTRADE country programs are the most comprehensive part of the Initiative. They identify opportunities and constraints for sustainable resource development in each country, focusing on bio-business development, bio-partnerships, sustainable use, conservation, and benefit-sharing incentives. Country program are managed by national focal points with long standing experience in the area of sustainable development and strong links with other national organizations. For example, the focal point in Colombia is the Humboldt Institute, and in Peru, the National Environmental Council (CONAM), while Guinée Ecologie, an NGO, is the Guinean focal point and Centre béninois pur le développement durable (CBDD) is the lead agency in Bénin.

The BIOTRADE Initiative thus aspires to fully integrate the private sector, government agencies, local and indigenous communities, and other relevant players in a mutually beneficial framework. It develops concrete partnerships with governments, private sector, international organizations and NGOs.

Suggestions on the focus of future long-term assessments:

Development on the agricultural and pharmaceutical sectors relies immensely on the access and utilization of new genetic resources for innovations that ultimately lead to new products. Recent developments in biotechnology are not only increasing demand in these sectors, but are also opening up new applications and markets for genetic resources. This has led to rapidly increasing demand for genetic resources, many of which are found in tropical forests.

It is important to understand the value placed by various stakeholders in the various forms of the exploitation of NTFPs. Given that the benefits (and costs) of exploitation are complex, interrelated and global, it is equally important to adopt a systematic methodology in establishing these benefits and costs flows of NTFPs. At the moment, many studies (including the pilot study by BDCP in 1997) used cross-sectional data to value NTFPs. The experience is that rarely have researchers embarked on time-series data in their economic valuation of biological diversity in general, and NTFPs in particular.

Given the preference for market-based policies around the world, and the theory, that for any resource to be properly managed, the cost of using the resource needs to reflect the values that society places on it. There is need to adopt reliable methodologies that would provide a reliable basis for policy action in respect of natural endowments.

We believe that generation of time-series data carried continuously and over a period of years will not only address relevant issues, but will also fill data gaps inherent in cross-sectional data, and therefore add to existing data pool.

1. Objectives of the study

1. Evaluate exploitation and uses of specific NTFPs in the communities over a period of 12 months, in the first instance. This will be used to establish the basis for subsequent time series study.
2. Determine market and non-market values of the species
3. Determine values that indigenes place on the NTFPs and forests in general
4. Assess market structure and marketing channels or specific NTFPs locally and/or across border.

2. Methodology and nature of data

The first phase consists of data generation to fill data gaps in the two communities (Imo and Ebonyi) where the study was carried out in 1997. Data gaps for 1998 and 1999 may be filled through projections, while 2000 and 2001 data could be established through memory recall, using household data for specific products.
Subsequently, multiple visit approach will be conducted in 6 communities. We are proposing Taraba, Cross River, Abia and Rivers states, in addition to the two former states (Imo and Ebonyi).

A semi-structured questionnaire will be designed to elicit information from households in the following areas: products/plant species usually collected, revenue generated from such products, gender issues with respect to access and use of products, knowledge levels of NTFPs, marketable and non-marketable value of plant species, market structure of specific products.

Data on three specific forest products, including bush mango, *Gnetum sp.* and *Afromomum sp*, will be collected with respect to product type, usual period of collection, quality and price of product.

3. Time Frame

Data collection will be for one year in the first instance, with plans to continue during the second year. Specific data will be collected on weekly basis. This implies four entries in a month.

4. Enumerators

We propose to employ one enumerator for each community. Thus, there will be 6 enumerators for the six states. Contact persons will be used in states where BDCP has already established some.

5. Sampling size

For more in-depth study, we propose 15 households per state, totaling 90 in all. We may need to segregate between households with medicinal knowledge (medicine men) and households without plant knowledge on the one hand, and female headed and male-headed on the other. Data will also be obtained from local medicine men.

6. Data Management

Data collection and entry will be regular. To facilitate this, we need to hire the services of a data analyst. Quarterly evaluation and reporting of data will be done to establish trends, identify lapses and gaps and modify data collection, if need be.

3.4 Computerized Information System for African Medicinal and Aromatic Plants (CISAMAP)

This report is organized as follows. In section 1, we give review of work done during the current of this past year that is year 4 (September 30, 2001 - September 30, 2002) of ICBG program. Section 2 provides plans for year 5 (September 30, 2002 - September 30, 2003). Section 3 presents future work plan, sustainability plan and wrap up of the current program.

In the next section, we present the progress on the database application development, the Interactive CD-ROM creation and the dynamic web site development. For each segment, we will outline the status at the end of last year (September 30, 2000 - September 29, 2001) and work performed this year (September 30, 2001 - September 29, 2002).

3.4.1 Database Application Development

1.1. Situation at the end of last year

Last year, we had proposed to unify the four databases (AFRICMED, ICBG-WRAIR, BIOMON and KFDP) in one ORACLE database called CISAMAP as shown in figure 15. Now, a user can choose to work with the whole database, or just one of its subset (a view). For example, if one is interested only in information related to medicinal plants, the view ICBG-WRAIR will be projected. For someone desiring only information related to chemical and biological aspect of plants, BIOMON view would be projected. The view KFDP will be projected if only demographic or geographical information is needed.

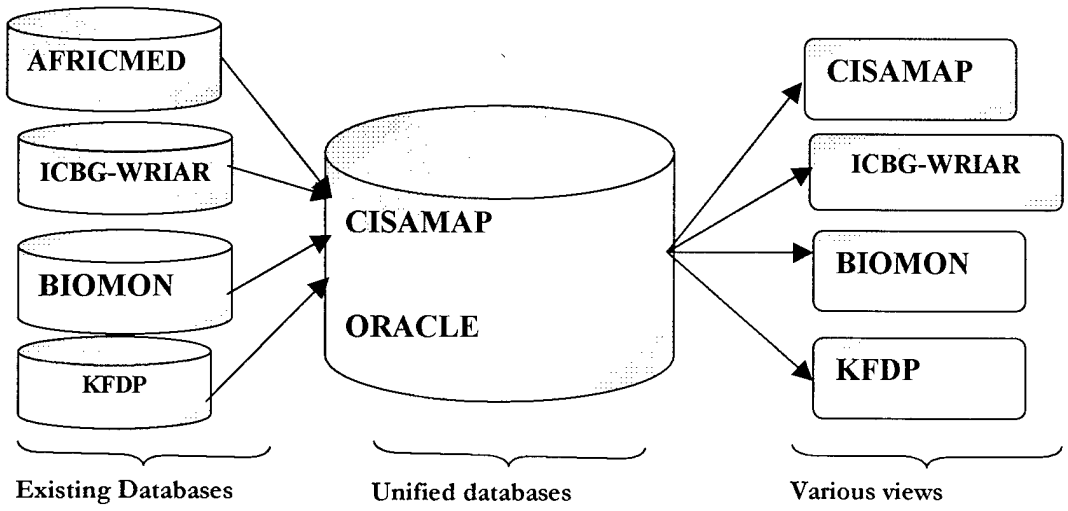


Figure 15: New Database System architecture

This structure allows any group working on a similar theme to import our system with few or no modifications. To reach this general structure, which increases the range of users of the system, we had to consider information related to:

- Medicinal plants (here stands for a medicinal plant or an animal since part of animal are also used in herbal medicine),

- Potions or herbal medicines (natural medicine), and
- Diseases as represented by traditional healers that treats them.

At the end of last year, we had finished conceiving and designing the ORACLE database entity tables. The coding and implementation using ORACLE SQL were quite advanced and we were waiting for the ORACLE System to progress normally. We had provided a detail description of the database structure in our last year report.

1.2 Work performed this year

To implement an ORACLE database there are four important phases:

a. *Phase1 (Definition of objectives)*: In this phase the objective is defined and the type of information to be contained in the database is determined. This task was performed last year.

b. *Phase2 (Definition of database schemas)*: A Database schema is a collection of structures of *tables, triggers, indexes, views, stored procedures, clusters, programmer defined objects (new types of data), tablespaces, partitions*, etc. Phase 2 is the main phase in creating an ORACLE database. It is here that all objects that will be manipulated in the database are defined. These definitions necessitate the following steps:

- Definition of tablespaces: A tablespace logically regroups data on a specific subject. One or more physical file(s) can be associated to each tablespace.
- Definition of tables: After defining the tablespaces, the definition of tables follows by defining the entities of the database. Each entity corresponds to a table of the database.
- Definition of users: Different users of the database are defined with appropriate rights.
- Definition of relations: Different links existing between the data and between the tables are determined. If necessary, new fields are added to tables or new tables are created to clarify the links in order to satisfy the integrity constraints and data coherence.
- Definition of indexes: New structures that will permit rapid access to various element of information are created.

c. *Phase3 (Creation of sequences)*: Theses sequences of numbers are used to populate primary keys of tables in the database. At this phase, the sequences are generated.

d. *Phase4 (Verification and Finishing)*: This fourth phase consists of testing the behavior of the database with real data. If necessary, the database is updated as well as the database schemas.

Sixty percent (60%) of phase 1 and phase 2 were accomplished last year. By the end of December 2001, we had completed the two phases. At the end of March, 2002, we were at 70% through phase 3. Today, we have completed phase 3 and are at 90% through phase 4. At this level of phase 4 (last phase of database system), we are in the process of installing the system (database and its interface: MEDITRA SOFTWARE) at designated sites to start the validation process.

2. User Interface (MEDITRA SOFTWARE)

2.1 Situation last year

Last year we also proposed a unified approach in specifying and modeling the database user interface that we named « MEDITRA SOFTWARE ». From that model, a user who wants to deal only with plants for its botanical proprieties for example will only receive two subsections of the interface: the subsection related to "source organs" and the one related to "Administration". The

interface was specified bilingual (French and English versions). A database populated using any of the two languages can be managed or consulted in either language. We had provided a detailed description of the specification of the system interface. At the end of last year 40% of the coding was done.

2.2 Work performed this year

Now, MEDITRA SOFTWARE system is fully implemented and is operational. Being application software and not a standardized software like Microsoft word, users must be trained to know how to use it properly and efficiently. This process will commence on completion of installation. As we mentioned in our last year report, MEDITRA SOFTWARE is designed to be used by several specialists in ethnobiology. It is thus necessary, when installing the software, to adapt it to the specific needs of each user by removing irrelevant information or procedures, or by adding new information or procedures not present. We have done 40% of the write up of user manual and technical manual.

3. Interactive CD ROM

3.1 Situation at the end of last year

As in the case of database organization, we had used a demo version to start developing a prototype with no video since we were still waiting for the video acquisition tool. The entire sequencing specification of the proposed CD-ROM was presented last year.

3.2 Work performed this year

We started the work with Authorware Professional. More than 90% of all the links specified are implemented. We are in the final stage of filling in all the multimedia data to check that the links implemented are functioning properly. So far, we have integrated textual data on Cameroon and a videotape on ICBG program in Nigeria. Additional video and audio data on Cameroon has been received to populate the Interactive CD-ROM. One of the key data is table of plants with their images, textual description of their uses and possibly video clip demonstrations of these uses.

In the mean time, we propose to combine video clips derived from a digitalized video film on Cameroonian traditional medicine, which is in color, and the black and white video clips from the videotape of ICBG, to fill the Interactive CD-ROM. The Interactive CD-ROM is bilingual (English and French) and is composed of four parts: ETHNOMEDICINE, ETHNOBOTANY, REGION, and REFERENCE. Presently, 80% of all the links implemented are working.

4. Online Server

4.1 Situation at the end of last year

We were asked to implement a Dynamic Web Site allowing consultation and updating of the database from any Internet Navigator such as Netscape Communicator or Internet Explorer. The Tools required for that task is Oracle WebDB. We were not able to start this activity because the demo version of Oracle WebDB in our possession could only run with ORACLE 8i.7 or later version that we did not have. At that time we were working with a demo version of 8i.5.

4.2 Work performed this year

Now that we have the correct version of Oracle (9i), we have assigned the two students we recruited on this part of the project. They started the work in July. We plan to combine Oracle WebDB and DREAMWAVER to produce attractive pages.

5. Conclusion

The database system, we have implemented, as well as its user interface will allow any organization working on a similar topic, to import our structure to generate its own database. The system comprises two components: an empty database and MEDITRA SOFTWARE (the interface to the database). MEDITRA SOFTWARE provides tools needed to populate an empty database. Work on the Interactive CD-ROM is progressing slowly because mounting video clips with Adobe Premiere is time consuming. 80% of links specified and implemented are working properly with the already filled in multimedia. The insufficiency in human resources forced us to postpone the starting date of the dynamic web site coding.

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