



International Organization for Chemical  
Sciences in Development

**Working Group on Plant Chemistry**

**CHEMISTRY, BIOLOGICAL AND  
PHARMACOLOGICAL PROPERTIES OF  
AFRICAN MEDICINAL PLANTS**

Proceedings of the first International IOCD-Symposium  
Victoria Falls, Zimbabwe, February 25-28, 1996



Edited by

**K. HOSTETTMANN,  
F. CHINYANGANYA,  
M. MAILLARD and  
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African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)  
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## 12. Development of ethical phytomedicines for Togo, West Africa

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### Introduction

The use of traditional remedies based on plant, animal and mineral extracts is a common practice in West African countries, especially in the rural areas where approximately 70-80% of the population lives. In Togo for example, ethnobotanical surveys indicate uses for >350 species of plants, 24 species of animals and 5 mineral preparations. (Adajanooun *et al.* 1996). The remedies are prepared by healers, according to their ancestral knowledge. The healers are well known in their villages and the population has a great regard for most of them. In Togo, they often have their own clinics and are well organized into a national association.

It is very common to see in West Africa, people using both modern and traditional drugs together. A survey of community attitudes in Ghana indicated that many patients believe that "a complete treatment consists of both" and that "modern drugs give fast relief but herbal remedies act slowly and provide the final cure" (Le Grand and Wondergem 1990). The great diversity of plant resources in West Africa is a great natural resource that can provide health care products locally at a fraction of the cost of modern pharmaceuticals. Unfortunately most traditional drugs have not been scientifically tested and physicians with western training are hesitant to recommend them even though they recognize their potential. This is because biological efficacy and toxicological tests may not have been performed with the traditional drugs or the mode of action and concentration of active principles is not known.

### Development of ethical phytomedicines

We are now attempting to bridge the gap between traditional and modern medicine by verification of selected medicinal plants as "ethical phytomedicines" in order to provide low cost medicines for the primary health care system in Togo. The different stages of development of an ethical phytomedicine are outlined in Table 12.1.

Table 12.1. Stages in development of ethical phytomedicines

- 
1. Ethnobotanical surveys
  2. Priority of medicinal plants according to the list of pathologies
  3. Review of the literature
  4. Botanical studies: collection, certification, vouchering and culture of the plant material, proper storage and stabilization to retain activity
  5. Mode of action studies, isolation of active principles and toxicology
  6. Galenic processing
  7. Clinical trials, drug authorization, marketing and pharmacovigilance
- 

#### *Ethnobotanical surveys*

Primary surveys of medicinal plants usage have been completed in Togo (Adajanhoun *et al.* 1996) and some neighboring West African countries (Iwu 1995). However, detailed knowledge of formulations and administration of effective remedies are lacking and require further ethnobotanical work. In West Africa, ethnobotanists must fully gain the confidence of healers who are reluctant to reveal details of species composition, preparation and administration of remedies.

Although it is a commonly held view that ethnobotany is often the best approach to drug discovery, there is little in the way of statistical data on the success of this approach. To test the ethnobotanical approach, we recently completed a quantitative analysis of 17 traditional malaria remedies and 12 control plants that showed that malarial remedies were more significantly active in bioassays than controls (Leaman *et al.* 1995). Local consensus among healers regarding malaria remedies, measured by an importance value index, was also highly predictive of those malaria remedies, with the highest activity in bioassays against *Plasmodium falciparum*.

#### *Priority medicinal plants*

The Ministry of Public Health in Togo has established a priority list of pathologies for which drugs are needed. These priorities include malaria, diabetes, diarrhea

and hypertension. Plants that have been selected for investigation are traditionally used for these pathologies. A list of some of the more commonly used plants, their pathologies and phytochemical markers are given in Table 12.2. Species of more interest from a drug discovery point of view are expected to be found in primary and secondary forests and savannah areas, rather than the well studied pan-tropical weed flora. Unfortunately, those natural areas that are the best sources of phytochemical resources are also areas most threatened by human activity. We are therefore collaborating with the IUCN to develop a rescue strategy for biodiversity resources.

Table 12.2. Commonly used plants with potential for development

Medicinal Plant	Use	Phytochemical marker
<i>Psidium guajava</i> (guava, goyavier)	diarrhoea	6-gingerol
<i>Zingiber officinale</i> (ginger, gingembre)	nausea	quercetin glycosides
<i>Carica papaya</i> (papaya, papayer, adibati)	antihelmintic	benzyl isothiocyanate
<i>Euphorbia hirta</i> (spurge, notsigbe)	antiamoebic	ellagic acid
<i>Momordia charantia</i> (Bitter melon, aduka)	diabetes	mormordicins, charantin
<i>Azadirachta indica</i> (neem, nim, margose)	antimalarial	gedunin
<i>Picrilima nitida</i>	antimalarial, antileishmanial, antitrypanosomal	akuammine
<i>Alstonia boonei</i>	antipyretic, antimalarial	echitamine
<i>Lippia multiflora</i> (thé de Gambie)	gingivitis	monoterpene profile
<i>Piper guineense</i> (Guinea pepper, poivrier)	hypotensive	piperine

### Review of the literature

Using the Natural Product Alert (NAPRALERT) database we have located and reviewed the primary literature with respect to priority plants (Awang and Arnason 1996). Two species, namely ginger and guava, have been the subject of sufficient investigation to be considered currently as ethical phytomedicines, because of the availability of clinical and toxicological data in addition to efficacy and phytochemical data. Ginger has been reviewed elsewhere and guava is considered in detail in a following section of this article. Other species in Table 12.1. may be advanced to ethical phytomedicines (or rejected) more rapidly than others not included here because of the considerable phytochemical and pharmacological information available. For example, the current state of our investigations of neem are also considered in this paper.

### *Botanical studies*

Identification of chemotypes and influence of environmental conditions on actives can be made more predictable by the application of chemical ecology principles to botanical studies. For example, Koumaglo's group has found that *Lippia multiflora* expresses at least three chemotypes with distinctly different profiles of volatiles present in the essential oil. Another critical point is post harvest treatment, since many secondary metabolites are unstable if not handled properly.

### *Mode of action, phytochemical and toxicological studies*

The first concern here is not the isolation of active principles but to confirm or reject in appropriate bioassays the indications of the remedy in traditional medicine. When the total extracts confirm these indications, the next steps are the activity guided isolation of the active principles and the study of the toxicology of the extracts. A priority for toxicology testing laboratory is to control environmental conditions to meet international standards.

### *Galenic processing*

The phytomedicine may be presented as an infusion, syrup, lotion etc., as required by therapeutic indications, but many plant materials may be delivered as a powder or extract in gel capsules or as a tablet. Standardization of dose at this stage is essential and depends on the identification of a suitable phytochemical marker (Marles *et al.* 1992), preferably, the active principle, as described in Table 12.2. The structures of several of these markers are illustrated Figure 1. We have recently developed or adapted straightforward HPLC procedures for the determination of 6-gingerol in ginger, quercetin in guava and gedunin in neem, which are operational in the Laboratoire des Extraits Végétaux et Arômes Naturels, Université du Bénin. Other analyses are readily accessible if pure standards can be obtained. Another quality control issue addressed in the Laboratoire de Microbiologie, Université du Bénin, is monitoring plant material for microbial contamination.

### *Clinical trials, approval, marketing and pharmacovigilance*

Clinical trials are the critical step towards approval of an ethical phytomedicine and along with the biological and phytochemical studies are the basis for approval by the appropriate body in the Ministry of Health. After release, a workshop for pharmacists and medical doctors is planned during which the results of studies will be presented along with the new drug. Following release of the product, a



pharmacovigilance committee will monitor any problems associated with the drug.

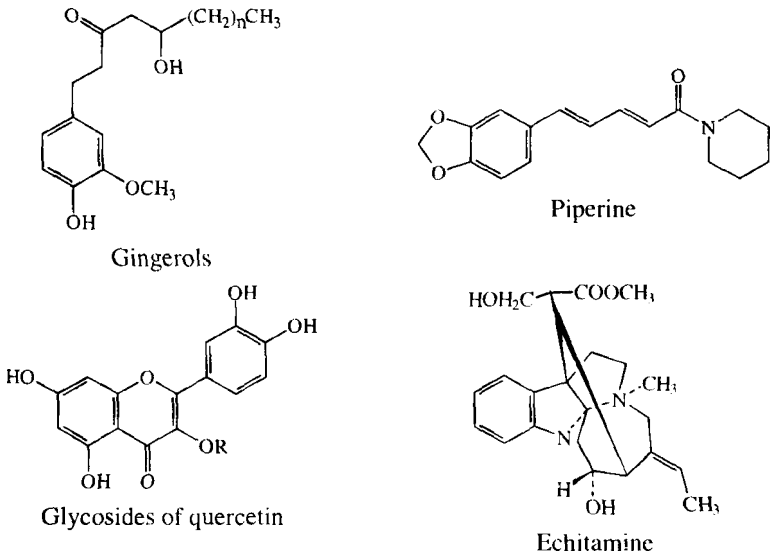
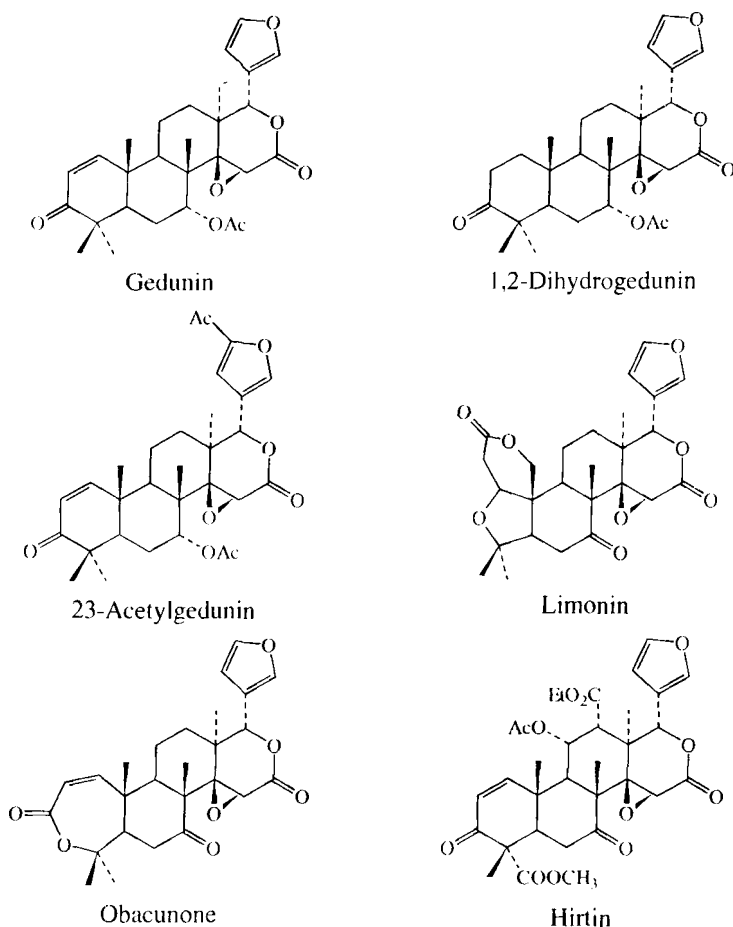


Fig. 12. 1. Phytochemical markers for standardization of phytomedicines.

### Guava leaf as an example of an ethical phytomedicine

Guava (*Psidium guajava*, Myrtaceae) is a low cost prophylactic for acute diarrhoea (AD) that has been used world-wide in traditional medicine. AD is a leading cause of mortality in children under two in developing countries. In Togo, guava leaves are used traditionally to prepare a decoction that is administered orally for AD. Following toxicological studies and clinical use in Mexico, Instituto Mexicano de Seguridad Social (IMSS), has given approval to this herbal remedy as a replacement therapy for more costly pharmaceuticals. Guava leaf extracts have antimicrobial activity to a variety of pathogens associated with AD (Iwu 1995). In addition, Lutterodt (1989) demonstrated that alcoholic Soxhlet extracts of guava leaf inhibited both the spontaneous and electrically coaxially stimulated contraction of isolated guinea pig ileum. These results suggested that the antidiarrhoeal activity of the extract is in part due to an inhibition of peristalsis. The inhibition is similar to that observed with morphine, one of the most effective clinically used drugs for treatment of AD.

In our phytochemical studies, a bioassay guided isolation of active fractions was undertaken and the active fractions analysed. Fourteen fractions were separated on a polyvinylpyrrolidone column using a water-methanol gradient. The greatest



**Fig. 12. 2.** Representative limonoids and derivatives tested.

inhibition of guinea pig ileum was found with two fractions which contained only flavonol glycosides. The compounds were identified as quercetin-3-O- $\alpha$ -L-arabinoside (guaijavarin); quercetin-3-O- $\beta$ -D-glucoside (isoquercitrin); quercetin-3-O- $\beta$ -D-galactoside; quercetin-3-O- $\beta$ -L-rhamnoside (quercitrin) and quercetin-3-O-gentiobioside. The quercetin glycosides were found to be hydrolysed to the aglycone in the gut and the aglycone was the most active compound in the bioassay. Therefore we have recently developed a method for the standardization of guava leaf based on total hydrolysed quercetin concentration by HPLC which will be used as a basis for controlling dosage as an ethical phytomedicine in Togo.

### Development of an antimalarial phytomedicine from neem

*Azadirachta indica* A. Juss. (Meliaceae) is widespread in Africa and is commonly known as neem in English, nim or margose in French and kiniti in Ewe. In the traditional pharmacopoeia it is used as therapy for malaria and fevers, as well as diabetes, hypertension and other conditions. Previous studies have suggested the *in vivo* antimalarial activity of some neem extracts and neem derived principles (Khalid *et al.* 1986, 1989).

Our investigations of neem extracts from Togo, in collaboration with Pezzuto and Angerhoffer at the University of Illinois, Chicago, showed that they did indeed have good antimalarial activity, with  $IC_{50}$ 's below the threshold for promising extracts (20  $\mu\text{g/gm}$ ) for both chloroquine sensitive (D6) and chloroquine resistant (W2) clones of *Plasmodium falciparum* (MacKinnon *et al.* *Journal of Natural Products*, submitted). In fact, unrefined neem extracts from Togo were the most active of 60 extracts of 22 species of Meliaceae which we evaluated for antimalarial activity against *Plasmodium falciparum* (MacKinnon *et al.* 1996). The neem extract contained approximately 0.1% of the recognized active principle, gedunin as measured by HPLC. A related species of Meliaceae, *Cedrela odorata*, available as a plantation tree in West Africa, also contains gedunin in ethanol or toluene extracts and showed activity in the screen (Table 12.3.).

Table 12.3. Antimalarial potential of gedunin-containing extracts as determined with *Plasmodium falciparum* clones in culture

Species or standard drug	extract	$IC_{50}$ [ $\mu\text{g/mL}$ ]	$IC_{50}$ [ $\mu\text{g/mL}$ ]
		Clone D6	Clone W2
<i>Azadirachta indica</i>	leaf in ethanol	2.50	2.48
<i>Cedrela odorata</i>	wood in ethanol	3.88	3.26
<i>Cedrela odorata</i>	wood in toluene	9.29	2.77
chloroquine		$3.4 \times 10^{-3}$	0.101
quinine		$9.5 \times 10^{-3}$	$38.2 \times 10^{-3}$
mefloquine		$2.7 \times 10^{-3}$	$0.9 \times 10^{-3}$
artemisinin		$4.5 \times 10^{-3}$	$2.2 \times 10^{-3}$

Evaluation of the antimalarial potential of pure gedunin (Figure 2) demonstrated that this limonoid has considerably higher activity than extracts, with an  $IC_{50}$  of 39 and 20 ng/mL against the D6 and W2 clones, respectively. Gedunin was more effective than five related limonoids tested (data for limonin, hirtin and obacunone are shown in Table 12.4.). With the resistant clone, gedunin was more effective than chloroquine or quinine but less active than artemisinin and mefloquine. The  $IC_{50}$  for cytotoxicity of gedunin to KB cells was 2300 ng/mL, suggesting a selectivity of > 100 for the resistant parasite compared to the human

cell line. Although the limonoid hirtin has 20% as much activity as gedunin, the selectivity for the parasite compared to KB cells was only 5.

In an attempt to improve on the activity of gedunin, we prepared a series of 10 derivatives, two of which are illustrated in Figure 2 and Table 12.4. Investigation of the antimalarial activity of these gedunin derivatives did not yield a compound with a higher activity than gedunin but was useful in defining the structural features contributing to activity of the molecule (MacKinnon *et al. Journal of Natural Products*, submitted).

Table 12.4: Antimalarial IC<sub>50</sub> values of limonoids and standard antimalarials

Compounds	<i>Plasmodium falciparum</i>	
	Clone D6 IC <sub>50</sub> [ng/mL]	Clone W2 IC <sub>50</sub> [ng/mL]
Gedunin	39 (100) <sup>#</sup>	20 (100) <sup>#</sup>
1,2-dihydrogedunin	>10000 (<0.39)	>840 (2.38)
23-acetylgedunin	832 (4.69)	156 (12.8)
Obacunone	>10000 (<0.39)	>10000 (<0.0005)
Limonin	>10000 (<0.39)	>10000 (<0.0005)
Hirtin	173 (22.6)	96 (20.8)
Chloroquine	1.3 (3000)	29.5 (67.8)
Quinine	14.8 (264)	34.9 (57.3)
Mefloquine	7.5 (521)	1.4 (1429)
Artemisinin	1.8 (2170)	0.5 (4000)

<sup>#</sup>Toxicity relative to the potency of gedunin (=100%)

Because of the traditional use and widespread acceptance of neem extracts, we believe it may be feasible to develop neem leaf preparations which are standardized based on gedunin content measured by HPLC. Further *in vivo* testing and toxicology and clinical trials will be required before such a medicine can be considered.

### Intellectual property

The question of intellectual property rights is not fully resolved in Togo as in many West African countries. Medicinal plants are generally regarded as a community resource, but individual healers jealously guard their individual recipes. It is clear, however that benefits derived from phytomedicines should be shared with the traditional healers.

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