Pharmaceutical Biology
2000, Vol. 38. No. 1, pp. 40-45

1388-0209/00/3801-0040$15.00

INVESTIGATION OF MEDICINAL PLANTS OF TOGO FOR ANTIVIRAL AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Methanol extracts were prepared from 19 medicinal plants of Togo and, by means of standard laboratory tests, were analysed for antiviral and antibiotic activities. Ten of the 19 showed significant antiviral activity and all but two displayed antibiotic activity. Extracts of three species, Adansonia digitata *(the most potent),* Conyza aegyptiaca *and* Palisota hirsuta, *were active against all three test viruses (herpes simplex, Sindbis and pollovirus). The other seven, however, were more selective, showing activity against only one or two viruses. The antibiotic profiles varied considerably. The observation that each extract showed a distinctive permutation of target organisms suggests that different bioactive phyrochemicals are present in each species. Only two of the extracts were devoid of bioactivity.*

INTRODUCTION

Most of the inhabitants of Togo (West Africa) rely on medicinal plant preparations for the treatment of many diseases, including those of an infectious nature. The preparations may be obtained from healers, or they may be processed directly from the raw materials according to traditional methods.

The investigation of traditionally-used plants as a guide to biologically active extracts has been well established. A recent illustration of this approach was the finding of Leaman et al. (1995) that antimalarial plants used by the Kenyah Dyak people in Borneo were significantly more bioactive in *Plasmodium falciparum* assays than control plants without such a tradition.

As part of a collaborative research program between our groups, we investigated 19 commonly used plants from Togo for antiviral and antimicrobial activities.

Recent studies conducted in our laboratories, and in others, have revealed that traditional plant medicines from various parts of the world can provide a rich source of antiviral and antibiotic activities (Vlietinck & Vanden Berghe, 1991; Yip et al., 1991; Hudson, 1995; Taylor et al., 1995, 1996). This is also true for West African species (de Souza et al., 1993, 1995; Gbeassor et al., 1996). Such types of study have often been justified in the context of phytochemical leads for pharamaceutical development. In the African context, however, bioactive extracts can also be considered as "ethical phytomedicines'y, if appropriate phytochemical standardization and toxicology investigations are undertaken (Gbeassor et al., 1996). However, In order to optimize the detection of these bioactivities, it is desirable to use flexible test protocols that can be modified to suit the test materials under study (Hudson et al., 1994). In particular, since many bioactive phytochemicals are photosensitizers (Hudson et al., 1995; Towers et al., 1997), then the inclusion of light exposure is

Kei-words: Antimicrobial; antiviral; herpes simplex virus; medicinal plants; pollovirus; Sindbis virus, Togo.

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recommended; otherwise some promising activities could be missed. These factors were taken into account for the present study and consequently we found that many of. the extracts investigated in this preliminary study displayed antiviral and antibiotic activities.

MATERIALS AND METHODS

Plant Collections .

Plants were collected in Togo in _September 1996 and air-dried. 'Voucher specimens were deposited in the Herbarium, Botany Department, the Université du Benin, Lome, Togo. Identifications -were made by Dr. K. Akpagana. The common uses of these plants are summarised in Table 1.

Preparation of Plant Extracts

Dried specimens were transported to the University of B.C. Department of Botany for processing. Powdered samples (20 g) were soaked overnight in 500 ml methanol and filtered through Whatman # 1 filter paper. The filter was rinsed with another 500 ml methanol, and the combined filtrates were evaporated and freeze-dried. Each dry extract was redissolved in methanol to 100 mg/ml.

Antiviral Assays

The technique described is a modified form of procedures described in detail previously (Marles et al., 1992; Taylor et al., 1996).

Vero cells (monkey kidney cell line, American Type Culture Collection) were grown in Dulbecco's Modified Eagle Medium (MEM) containing 5% fetal bovine serum (all cell culture reagents were obtained from GIBCO Life Sciences, Ontario), In 96-well microtest trays (Falcon), 0.2 ml per well. When the. cells formed confluent monolayers, they were used for the assays.

Each plant extract was diluted 1:100 in MEM plus 0. I % serum (Hudson et al., 1994) and filtered through a sterile syringe filter 0.2 p pore diameter. The filtrate, equivalent to 1,000 pg/ml dried plant material and 1% methanol, was the starting test material.

In the standard procedure, serial 2-fold dilutions of the extract were made (in duplicate), In MEM + 0.1 % serum, across a row of wells in an empty 96-well microtest tray. With the aid of a multipipettor, these diluted extracts were transferred to the aspirated Vero cell monolayers of another 96-well tray, 0.1 ml per well. These cultures were incubated at 37°C for 60 min and examined microscopically for possible immediate cytotoxic effects. Then, 0.1 ml of virus (HSV, herpes simplex virus; SINV, Sindbis virus or poliovirus type 1; Taylor et al., 1996), comprising 100 pfu in MEM + 0.1% serum, was added to each well. Immediately, the tray was transferred to an environmental chamber (37°C) and exposed to a combination of visible light plus UVA (long-wave ultraviolet) for 30 min, with continuous gentle shaking of the tray. The lamps (fluorescent and BLB) were arranged to give approximately 5 watts/ m^2 incident radiation of both visible and UVA. After the light exposure, the trays were returned to the cell culture incubator. Controls included cells with no virus, and cells infected with untreated virus. Cultures were inspected periodically In the microscope for viral cpe (characteristic virus-induced cytopathic effects). In the case of HSV, complete cell destruction (100% viral cpe) required 4 days; for SINV, 3 days; for poliovirus, 2 days. Absence of cpe indicated complete inactivation of the 100 pfu of virus; substantially less than 100% cpe at the designated times indicated partial inactivation.

"Cytotoxicity" Assays

The procedure was similar to the antiviral assay. except that no virus was added to the wells, and following light exposure, the trays were returned to the incubator for periodic microscopic assessment of changes in cell morphology or visible toxic effect (obvious cellular damage or lysis).

Antibiotic Assays

The disk diffusion assay (Taylor et al., 1995) was used to test for antibacterial and antifungal activity, with the addition of UVA exposure. Filter paper disks (Schleicher and Schuell 740E) were impregnated with 20 pl aliquots (equivalent to 2 mg dried extract) of the methanol extracts. Test organisms were: *Staphylococcus aureus (Sa); Streptococcus fecalis (St), Bacillus subtilis (Bs), Escherichia* coli (Ec); *Klebsiella pneumoniae (Kp); Salmonella gphimurium (St); Pseudomonas aeruginosa (Pa); Mycobacterium phlei (Mp); Candida albicans (Ca).* Replicate culture plates were kept dark or exposed to UVA lamps for 2 h (Taylor et al., 1995). Zones of growth inhibition were recorded after 24 h incubation (48 h for Mp).

RESULTS AND DISCUSSION

Table I lists the plants used In this study, with information on their traditional applications. Many of them are used to treat infections as well as other disorders.

Table 1. Plants used in this study.

Family, genus species and authority	Part of plant used	Treatment
Apocynaceae		
Alstonia boonei De Wild.	leaf	liver complaint, malaria
Asteraceae		
Acanthospennum hispidum DC.	-leaf --.	malaria
Chrysanthellum senegalensis DC.	whole	kidney disease
Conyza aegiptiaca (L.) Aiton	leaf	skin disease, menstrual disorders
Eupatorium odoratum L.	leaf	fever, skin disease, prevent abortion
Tagetes patula L.	leaf	
Vemonia glaberrima Welw. ex O. Hoffm.	leaf	malaria
Bignoniaceae		
Spathòdea campanulata P. Beauv.	leaf	pain. wound, menstrual disorders
Bómbacaceae		
Adansonia digitata L.	leaf, root. bark	asthma
Commelinaceae		
Palisotà hirsuta (Thunb.) K. Schum.	leaf	diarrhoea. skin disease
Davalhaceae		
Davallia chaerophylloides (Poir.) Steud.	leaf	diarrhoea. wounds
Malvaceae		
Sida acuta Burm. f.	leaf	eczema, kidney stones, headache
Moraceae		
Ficus ovata Vahl	root, bark	tetanus convulsions
Orchidaceae		
Cahptrochilum emarginatum (Afzel.ex Sw.) Schhr.	whole	menstrual disorders
Rubiaceae		
Mitracarpus villosus (Sw.) DC.	leaf	skin diseases
Rutaceae		
Zanthoxvlum zanthoxvloides (Lam.) zepemick & Tímler	root, bark	wound, pain
Simarubaceae		
Harrisonia abvssinica Oliv.	leaf	diabetes, wound
Sapindaceae		
Paullinia pinnata L.	whole	blood pressure, aphrodisiac, diarrhoea
Verbenaceae		
Lippia multiflora Moldeake	leaf	liver complaints

The results of the "cytotoxicity" assays are shown in Table 2. This protocol, which involves continuous exposure of the cells to extract for 5 days, permits detection of cytotoxic effects that lead to cell death, as wen as more subtle effects on the cells that may not be deleterious, e.g., production of granules in the cytoplasm, alteration of cell shape to a more rounded morphology. Most of the extracts produced some such changes in cell morphology, although only eight were toxic at higher concentrations (> 500 pg/ml). In addition, one extract, *Lippia multiflora,* was cytotoxic at concentrations as low as 125pg/ml. Apart from this last-named extract, no others caused cellular changes that could interfere in the interpretation of antiviral tests.

Table 3 summarizes the results of the antiviral assays, which involved three different animal viruses (all of which can infect humans). Activity was found In 11/20 (19 species; and two types of extract from A. *digitata)* extracts. Three species, *Adansonia digitata, Conyza aegpytiaca, Palisota hirsuta,* affected all three viruses, with A. *digitata* being the most potent. Two extracts affected two of the viruses, and six affected only one virus. Among the viruses, HSV was the most commonly affected (11 extracts); polio and SEW were sensitive to five and four extracts, respectively. *Paullinia pinnata* was interesting by virtue of its impressive selective activity against HSV The other selective activities were shown by *Davallia chaerophylloides. Sida acuta, Zanthoxylum zanthoxyloides* and *Harrisonia abvssinica,* although they were not as potent as P. *pinnata.* However, from the point-of-view of medicinal plant applications, extracts with broad-spectrum activities and minimal cytotoxic effects might be more important.

Table 4 summarizes the antibiotic data. Most of the extracts had some antibiotic activities, although the spectrum of sensitivities varied. In addition certain activities required UVA, or were enhanced by UVA, indicating the presence of a photosensitizer. Several other bacteria, *Salmonella typhimurium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* and the fungal organism *Candida albicans,* were unaffected.

Table 2. Cytotoxicity assays.

Family. genus and species	Cytotoxicity (WgIml)	Cellular changes (pg/ml)
Apocynaceae		
Alstonia boonei	–	rg 1.000
Asteraceae		
Acanthospermumhispidum		
Chrysanthellum senegalensis	–	rg 500
Cony:a aegpptiaca	Soo	rg 500
Eupatorium odoratum	–	g 250
Tagetes patula	–	g 500. r 250
Vfrnonia gfaberrinm	*1.000*	g 500
Bignoniaceae		
Spathodea campanulata	–	g 1.000. r 500
Bombacaceae		
Adansonia digitata root bark	500	r 250
Jeaves	500	r 125
Cotnmefinaceae		
Palisota hirsuta	500	r 250
Davalliaceae		
Davallia chaerophylloides	–	-
Malvaceae		
Sida acura	500	rg 250
Moraceae		
Ficus ovata	500	r 250
Otchidaccae		
Caltiptrochilum emarginatum	–	g 1.000
Rubiaceae		
Mirracarpus villosus	–	*g 1.000*
Rutaceae		
Zanthosvlum ;anthoxvloides	–	r 500
Simasubaceae		
Harrisonia abvssinica	–	*r 1.000*
Sapindaceae		
Paullinia pinnata	*1.000*	g 500
Verbenaceae	125	
Lippia multiflora		

l minimum concentration causing effect
-' r. cells become rounded but remain attached; g, granules evident in cytoplasm
- no visible change

Table 3. Antiviral activities. I

Family. genus and species		Virus targets herpes simplex	Sindbis	Polio
Apocynaceae				
Alstonia boonei		*0*	*0*	*0*
Astetaceae				
Acanthospermum hispidum		*0*	*0*	*0*
Chrvsanthellurn senegalensis		*0*	*0*	*0*
Conyza aegypuaca		*1*	2	l
Eupatorium odoratum		*0*	*0*	*0*
Tagetes patula		*0*	*0*	*0*
Vernonia glaberrima		*0*	*0*	*0*
Bignoniaceae				
Spathodea campanulata		*0*	*0*	*0*
Bombacaceae				
Adansonra digirara	root bark	3	2	2
	leaves	4	2	0
Commelinaceae				
Palisota hirsuta		4	l	2
Davalliaceae				
Davallia chaerophrlloides		*1*	*0*	*0*
Malvaceae				
Sida acuta		*2*	*0*	*0*

Table 3 continues

Table 3 (continued)

Family, genus and species	Virus targets herpes simplex	Sindbis	Polio
Moraceae			
Ficus ovaia	3	0	2
Orchidaceae			
Cahptrochilum emarginatum	0	0	0
Rubiaceae			
Mitracarpus villosus	3	0	1
Rutaceae			
Zanthoxvlum zanthoaYdoides	1	0	0
Simarubaceae			
Harrisonia abvssinica	2	0	0
Sapindaceae			
Paullinia pinnata	3	0	0
Verbenaceae			
Lippia multiflora -	0	0	0

t Antiviral activity measured as complete or partial alleviation of viral cpe (cytopathic effects) at minimum concentration of 500 p;/ml (1). 250 pg/ml (2), 125 ILS/ml (3), 62.5 jig/ml (4). 0 = no detectable activity.

Table 4. Antibiotic activities.

Family, genus and species	Susceptible microorganisms t
Apocynaceae	
Alstonia boonei	none
Asteraceae	
Acanthospermum hispidum	none
Chnsaniltellum senegalensis	Sa'- Sf Ec (UVA only) Mp (UVA enhanced)
Com`a aeg3ptiaca	Sa Ec (UVA only) Mp
Eupaiorium odoratum	Sa Ec (UVA enhanced) Bs Mp
Tagetes patula	Sa Ec (UVA only)
Vernonia glaberrima	Sa (UVA only) Bs (UVA enhanced) Mp
Bignoniaceae	
Spathodea campanulata	Sa (UVA enhanced) Ec (UVA only)
Bombacaceae	
Adansonia digitata +	Sa Sf Bs Ec Mp
Commelinaceae	
Palisota hirsuta	Sf (UVA only) Sa Bs Ec
Davalliaceae	
Davallia chaerophvlloides	Sa (UVA only)
Malvaceae	
Sida acuta	Sa Bs Ec (UVA only) Mp
Moraceae	
Ficus ovata	Sa Sf Bs Ec Mp
Orchidaceae	
Calyptrochilum emarginatum	Sa
Rubiaceae	
Mitracarpus villosus	Sa Bs Ec
Rutaceae	
Zanthoxvlum zanthoxvloides	Ec (UVA only) Mp
Simarubaceae	
Harrisonia abpssinica	Sa Bs Ec Mp
Sapindaceae	
Paullinia pinnata	Sa Bs Ec Mp
Verbenaceae	
Lippia multiflora	Sa Bs Ec

t Indicated by zone of inhibition in disk assay

'-Abbreviations: Sa *Staphylococcus aurcus,* Sf *Streptococcus jecalis,* Bs *Bacillus subtilis, Ee Escherichia coli, Mp Mycobacterium phlei.* Organisms **resistant to all extracts were:** ***Salmonella typhimurium, Klebsiella pneun+oniae, Pseudomonas. aenginosa, Candida*** *albicans.*

Two extracts, *Alstonta boonei* and *Acanthospermum hispidum,* were devoid of antimicrobial activity as well as antiviral activity (Table 3). In general, there appeared to be a rough correlation between antiviral and antibiotic activities (in terms of range of target organisms). However, due to the qualitative nature of the antibiotic disk assay it is difficult to make conclusions about relative potencies.

We have no information on the nature of the bioactive phytochernicals, although It is worth noting that because of the presence of *so* many different combinations of microbial targets for different extracts, together with indications of photosensitizers In some cases, it would appear that different types of compound ate involved.

It is interesting to attempt to correlate the traditional applications of the plant extracts with the microbial targets. In some cases this is feasible, e.g., P. *hirsuta* and M. villosus are both used to treat skin diseases. Both of them were very active against herpes simplex virus, which causes skin infections, as well as some skin bacteria. However, such correlations were not apparent in other cases. This may indicate that some extracts contain other kinds of bioactive phytochemicals, in addition to or instead of direct-acting andvirals/antibiotics.

ACKNOWLEDGEMENTS

We wish to thank three Togolese healers for their help with information on plants: Amenoudji Dodji, Sessou Messanvi (deceased) and Ahyi Amakoue Michel. We also thank Zyta Abramowski for her assistance. This work was supported by CIDA (Canadian International Development Agency) Ottawa.

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Accepted: July 27. 1999