Journal of Ethnopharmacology 128 (2010) 501–512

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ethnobotanical survey and cytotoxicity testing of plants of South-western Nigeria used to treat cancer, with isolation of cytotoxic constituents from *Cajanus cajan* Millsp. leaves

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ARTICLE INFO

Article history:
Received 30 October 2009
Received in revised form
28 December 2009
Accepted 3 January 2010
Available online 11 January 2010

Keywords:
Ethnobotanical survey
Cytotoxicity
Cancer
Cajanus cajan
Stilbenes
SRB assay
XTT assay

ABSTRACT

Ethnopharmacological relevance: There is only scant literature on the anticancer components of medicinal plants from Nigeria, yet traditional healers in the area under study claim to have been managing the disease in their patients with some success using the species studied.
Aim of study: To document plants commonly used to treat cancer in South-western Nigeria and to test the scientific basis of the claims using *in vitro* cytotoxicity tests.
Methods: Structured questionnaires were used to explore the ethnobotanical practices amongst the traditional healers. Methanol extracts of the most common species cited were screened for cytotoxicity using the sulforhodamine B (SRB) assay in both exposure and recovery experiments. Three cancer cell lines (human breast adenocarcinoma cell line MCF-7, human large cell lung carcinoma cell line COR-L23 and human amelanotic melanoma C32) and one normal cell line (normal human keratinocytes SVK-14) were used for the screening of the extracts and the fractions obtained. The extract of *Cajanus cajan* showed considerable activity and was further partitioned and the dichloromethane fraction was subjected to preparative chomatography to yield six compounds: hexadecanoic acid methyl ester, α-amyrin, β-sitosterol, pinostrobin, longistylin A and longistylin C. Pinostrobin and longistylins A and C were tested for cytotoxicity on the cancer cell lines. In addition, an adriamycin-sensitive acute T-lymphoblastic leukaemia cell line (CCRF-CEM) and its multidrug-resistant sub-line (CEM/ADR5000) were used in an XTT assay to evaluate the activity of the pure compounds obtained.
Results: A total of 30 healers from S W Nigeria were involved in the study. 45 species were recorded with their local names with parts used in the traditional therapeutic preparations. Cytotoxicity (IC_{50} values less than 50 μg/mL) was observed in 5 species (*Acanthospermum hispidum*, *Cajanus cajan*, *Morinda lucida*, *Nymphaea lotus* and *Pycnanthus angolensis*). *Acanthospermum hispidum* and *Cajanus cajan* were the most active. The dichloromethane fraction of *Cajanus cajan* had IC_{50} value 5–10 μg/mL, with the two constituent stilbenes, longistylins A and C, being primarily responsible, with IC_{50} values of 0.7–14.7 μM against the range of cancer cell lines.
Conclusions: Most of the species tested had some cytotoxic effect on the cancer cell lines, which to some extent supports their traditional inclusion in herbal preparations for treatment of cancer. However, little selectivity for cancer cells was observed, which raises concerns over their safety and efficacy in traditional treatment. The longistylins A and C appear to be responsible for much of the activity of *Cajanus cajan* extract.

1. Introduction

There is currently a global renaissance of ethnobotanical surveys of medicinal plants and the need for screening specific parts of plants (Li and Vederas, 2009; Paterson and Anderson, 2005; Igoli et al., 2005). Although much screening of medicinal plants for potential anticancer activity has occurred in the last fifty years, the study of ethnopharmacological leads from African medicinal plants has not been realized as fully as from other traditional societies such as India and China. However the recent screening of 7500 species from South Africa (Fouche et al., 2008) marks some progress and the present work investigates some species from South-western Nige-

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doi:10.1016/j.jep.2010.01.009

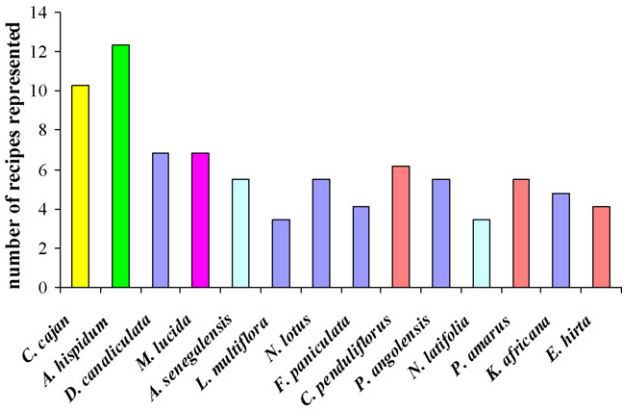

Fig. 1. Species frequency in the recipes commonly used for treatment of various cancer types in South-western Nigeria as evident from the ethnobotanical survey.

ria. The Nigerian government has recently set aside US$1billion for the development of traditional medicine and to encourage its integration at all levels of health care delivery system of the country (Adelaja, 2006).

In South-western Nigeria, traditional medicine is part of the cultural heritage, and is acceptable to the majority of the populace. However the secrecy attached to it has prevented much scientific recording of the knowledge and few literates have been taken into confidence. The present study investigates some plants from this area used to treat cancer by local healers, who divulged the information as part of a survey built on established trust.

2. Experimental

2.1. Ethnobotanical survey

The survey was carried out in four major states [Ogun, Oyo, Lagos and Ekiti] in the South-western region of Nigeria. This area contains savannah, mangrove and rain forest vegetations and has a diversity of ethnic groups including Awori, Egun, Egba, Ekiti, Eyo, Ijebu, Oyo, Yewa, and Yoruba.

Table 1
List of plants that are used in South-western Nigeria for treatment of cancer.

Botanical name	Local name(s) (Yoruba)	Voucher number	Family	Part(s) used	Mode of preparation and use (T-topical; O-drunk)
Acanthospermum hispidum D.C.	Dagunro, Dagunro	FHI106989	Asteraceae	Flowering shoots	Poultice (**T**); decoction (**O**)
Acanthus montanus (Nees) T. Anderson	Ahon-Ekun	FHI109031	Acanthaceae	Leaves and roots	Poultice (**T**)
Adenopus breviflorus Benth.	Tagiiri	FHI109040	Cucurbitaceae	Unripe fruit and leaves	Poultice(**T**)
Aframomum melegueta [Roskoe] K. Schum.	Ata-ire	FHI109051	Zingiberaceae	Fruiting shoot	Poultice(**T**)
Allium ascalonicum L.	Alubosa elewe	FHI109046	Liliaceae	Whole plant	Decoction(**O**); infusion(**O**)
Anchomanes difformis (Blume) Engl.	Agooni, Abirisoko	FHI109032	Araceae	Rhizome	Infusion(**O**); poultice(**O**)
Annona senegalensis Pers.	Abo	FHI106990	Annonaceae	Leaves	Infusion(**O**); decoction(**O**)
Cajanus cajan (L.) Millsp.	Otili	FHI106993	Fabaceae	Leaves	Poultice(**T**); concoction(**O**)
Canavalia ensiformis (L.) D.C.	Sese-nla	FHI109033	Papilionaceae	Seeds	Poultice(**O**); decoction(**O**)
Croton penduliflorus Hutch.	Aworo-oso	FHI106988	Euphorbiaceae	Seed and stem bark	Decoction(**O**)
Croton zambesicus Muell. Arg.	Ajeofole	FHI109041	Euphorbiaceae	Stem bark	Decoction(**O**); concoction(**O**)
Curculigo pilosa (Schum. and Thonn.) Engl.	Epa-ikun	FHI109047	Hypoxidaceae	Rhizome	Infusion(**O**)
Cymbopogon citratus (D.C.) Stapf	Ekan	FHI109052	Poaceae	Leaves and rhizome	Concoction(**O**)
Dioclea reflexa Hook. f.	Ebe, Agbarin	FHI109054	Fabaceae	Seeds	Poultice(**T**)
Dioscorea hirtiflora Benth.and Hook.	Isanyinahun	FHI109034	Dioscoreaceae	Leaves	Decoction(**O**)
Diospyros canaliculata De Wild.	Oriloje, Odubu	FHI106994	Ebenaceae	Leaves	Decoction(**T**, **O**)
Entandrophragma macrophyllum A. Chev.	Arunje, Ijebo	FHI109042	Meliaceae	Stem bark	Decoction(**O**)
Euphorbia heterophylla L.	Oro	FHI109053	Euphorbiaceae	Root and leaves	Decoction(**T**)
Euphorbia hirta L.	Emile, Oro-elewe,	FHI109062	Euphorbiaceae	Shoot	Concoction(**O**, **T**)
Euphorbia laterifolia L.	Enu-kopire	FHI109056	Euphorbiaceae	Root and leaves	Infusion(**T**)
Euphorbia poissonii L.	Oro-adete	FHI109035	Euphorbiaceae	Sap	Poultice(**T**)
Flabellaria paniculata Cav.	Lagbolagbo	FHI106996	Malpighiaceae	Leaves	Decoction(**O**)
Funtumia africana (Benth.) Stapf.	Ako-ire	FHI109039	Apocynaceae	Stem bark	Decoction(**O**)
Imperata cylindrica (L.) Räusch.	Ekan	FHI109045	Poaceae	Root	Decoction(**O**)
Jatropha curcas L.	Botuje, Lapalapa	FHI109036	Euphorbiaceae	Root and stem bark	Decoction(**T**, **O**)
Jatropha gossypifolia L.	Botuje-pupa	FHI109050	Euphorbiaceae	Root and stem bark	Decoction(**T**, **O**)
Kigelia africana (Lam.) Benth.	Pandoro	FHI109060	Bignoniaceae	Fruit	Decoction(**T**, **O**)
Lippia multiflora Moldenke	Eforomoba	FHI106995	Verbenaceae	Leaves	Poultice(**T**)
Luffa cylindrica (L.) Roem.	Kankan-ayaba	FHI109037	Curcurbitaceae	Fruit and leaves	Infusion(**T**)
Mezoneuron benthamianum Baill.	Jenifiran, Ekanan-Ekun	FHI109058	Caesalpinaceae	Root	Decoction(**O**)
Microdesmis puberula Hook. f. ex Planch	Esunsun	FHI109038	Euphorbiaceae	Root	Decoction(**O**)
Mitragyna inermis (Willd.) K. Schum.	Okobo	FHI109049	Rubiaceae	Stem bark	Decoction(**O**)
Morinda lucida Benth.	Oruwo	FHI106992	Rubiaceae	Leaves and stem bark	Infusion(**O**); decoction(**O**)
Nauclea latifolia Smith	Ira	FHI109044	Rubiaceae	Root and leaves	Decoction(**O**)
Nymphaea lotus L.	Osibata	FHI106987	Nymphaceae	Leaves	Decoction(**T**, **O**)
Olax subscorpioidea Oliv.	Ifon	FHI109065	Olacaceae	Root	Decoction(**O**)
Phyllanthus amarus Schumach. and Thonn.	Ajelara	FHI109059	Euphorbiaceae	Shoot	Decoction(**T**)
Piptadeniastrum africanum (Hook.f.) Brenan	Agboin	FHI109043	Mimosaceae	Root	Decoction(**O**)
Pycnanthus angolensis (Welw.) Warb.	Akomu	FHI106991	Myristicaceae	Stem bark	Poultice(**O**)
Securidaca longipedunculata Fres.	Ipeta	FHI109048	Polygalaceae	Root	Decoction(**O**, **T**)
Triplochiton scleroxylon K. Schum.	Arere	FHI109063	Sterculiaceae	Stem bark	Decoction(**T**)
Vernonia amygdalina L.	Ewuro	FHI109061	Compositae	Root	Decoction(**O**)
Xylopia aethiopica (Dunal) A. Rich.	Eeru	FHI109057	Annonaceae	Fruits	Decoction(**O**); poultice(**T**)
Zanthoxylum zanthoxyloides Zepern. and Timler	Ata	FHI109064	Rutaceae	Stem bark and root	Decoction(**O**)
Zingiber officinale Roscoe	Ata-ile	FHI	Zingiberaceae	Rhizomes	Concoction(**O**), decoction(**O**)