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Ophioglossum polyphyllum A. Braun in Seub. (Ophioglossaceae, Pteridophyta), a rare potherb in south central Tibet (T.A.R., P.R.China)

Ophioglossum polyphyllum A. Braun in Seub. (Ophioglossaceae, Pteridophyta), une herbe potagère rare du Tibet centro-méridional (Région autonome du Tibet, R.P. Chine)

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Résumé : L'article rassemble des données relatives à la connaissance ethnobotanique (herbes potagères) des Ü du Tibet centro-méridional. Une fougère comestible, *Ophioglossum polyphyllum*, est reconnue et sa composition chimique est présentée. Sa valeur alimentaire est discutée.

Mots clés : Tibet, nutrition alternative, herbe potagère, Pteridophyta, composition chimique

Abstract: The paper set up data dealing with potherbs knowledge of Ü of South Central Tibet. An edible fern, *Ophioglossum polyphyllum*, is recognized and its chemical composition presented. Its food value is discussed.

Keywords : Tibet, alternative foods, potherbs, Pteridophyta, chemical composition.

INTRODUCTION

South Central Tibet is primarily inhabited by Ü people. Four main ecological units are easily recognised from urban ecosystem, to peri-urban area, rural environment and high altitude grasslands undergoing cattle by nomads.

Few information is available regarding the food intake of peasants in the rural area of South Central Tibet. Nevertheless an environmental approach was recently published (HAUBRUGE et *al*., 2000), as well as preliminary information on landscape perception and diversity of alternative foods (MALAISSE et *al*. 2003). From a general approach, alimentation of Ü peasants as been perceived as monotone, based primarily on barley, with few "legumes" (cabbages, onions) and only some grams of meat.

Moreover the general poverty of soils in minerals, local deficiencies in Se, importance of runoff, conditions that prevail also on Andes high plateaux and in the Alps should be remind.Sanitary health indicates less nutritional deficiencies at the beginning of autumn than at the coming out of winter, a period when notably more stomatites are observed [vitamin deficiencies, notably B 12 (GOYENS,pers. com.)].

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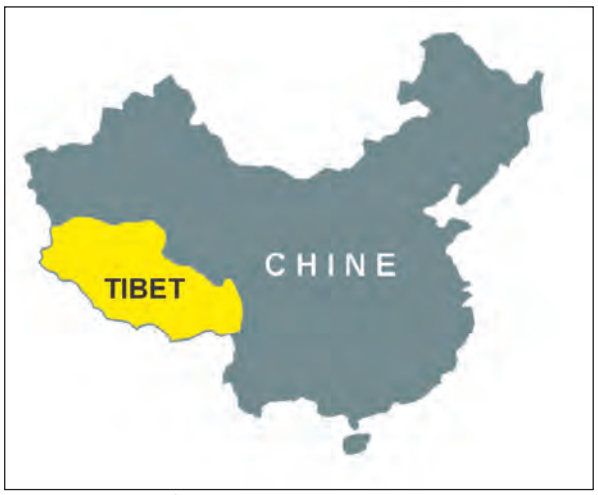


Fig. 1: carte de situation

Therefore, giving interest to alternative foods appear justified (MALAISSE 2001) and made the aim of an ethno-ecological survey conducted from 2000 to 2005 during Summers, and in Autumn 2007, successively in the frame of the Kashin Beck Disease Project (HAUBRUGE et *al*. 2000) of "Médecins Sans Frontières-Belgium" (MSF-B), and later in the frame of the Kashin-Beck Disease Foundation.

During this survey the importance accorded by local populations in a restricted area of Netong county to a rare potherb, a fern (adder's-tongue) known locally as "tuchung", became obvious. The present note investigates the nutritional value of this rare food.

PTERIDOPHAGY

Pteridophyta were mostly considered as "ferns and fern allies". From a systematic point of view, they comprise true ferns (Filicales) as well as a special group of closely related plants, such as whisk ferns (Psilotaceae), clubmosses (Lycopodiaceae), spikemosses (Selaginellaceae) and horsetails (Equisetaceae). A recent study indicates that Lycophytina have to be excluded from Euphyllophytina, whilst Psilotidae are closely related to Ophioglossidae (PRYER et *al*. 2001).

Consumption of ferns and ferns allies has been reported by several sources (MAY 1978, COPELAND 1982, JONES 1987). Six main items are looked for food : crosiers, tree starch, underground tubers, rhizomes, gemmae and sporocarps. In several countries, crosiers of unopened fronds or « fiddleheads » of ferns are eaten as green vegetables or even constitute a staple diet (CHRISTENSEN 1997, 2002). They have mostly to be braised, or roasted to remove shikimic acid (ISAACS 1994).

Firstly fern tips are reported to be eaten both in North (NORTON 1979, von ADERKAS 1984) and South America (BARFOD & KVIST 1996), as in Europe (WEE 1997), Africa (MALAISSE 1997), Asia (PIGGOTT & PIGGOTT 1996, WEE 1997) and Oceania (ISAACS 1994, WEE 1997). Bundles of freshly uncurled fronds are a common sight in the rural markets of the Philippines, Indonesia, Malaysia and New Guinea (MALAISSE 2007). Moreover, some restaurants in South-East Asia offer ferns as an exotic vegetable dish, when supplies are available (WEE 1997, MALAISSE 2007). Secondly, the soft pithy tissue near the top of the trunk of several tree ferns (Cyatheaceae) contains a lot of starch that can be eaten either raw or cooked (Isaacs 1994, Wee 1997). In the past, starch of tree ferns was eaten in India, New Zealand, the Philippines, Madagascar, New Guinea, New Caledonia, Hawaii and Australia (WEE 1997). Moreover some tubers are also eaten; between others those of some Nephrolepidaceae (CUYPERS 1970). The consumption of rhizomes as well as of gemmae, the adventitious buds arising on the fronds of some species, has rarely been quoted (BARFOD & KVIST1996). Lastly sporocarps, such as those of Marsileaceae, are also reported to be eaten (CHERIKOFF 1993).

MALAISSE (2007) listed more than hundred edible ferns. According to WEE (1997), *Diplazium esculentum* (Retz.) Sw. is the most popular ferns green in South East Asia, because it is the tastiest of them all. In Sarawak, ferns are a popular side dish, amouting locally about 10% of all vegetable dishes eaten (CHRISTENSEN 1997). The consumption of roasted crunchy jungle ferns, such as "midin" in Malaysia, is even strongly suggested by tourism guides (ROWTHORN et *al*. 1999).

Within the edible ferns, four belongs to the Ophioglossales order. Indeed, *Ophioglossum reticulatum* L. is used as a substitute of spinach in villages and towns of Uttar

Pradesh and Madhya Pradesh in India (SHARMA 2000), being still appreciated in Indonesia were it is eaten as salad or cooked as a vegetable. In Nepal, its fronds as well as those of *O. nudicaule* L. are cooked as a vegetable (MANANDHAR 2002). On the other hand, the fronds of *Helminthostachys zeylanica* (L.) Hook. are eaten as a salad in rural areas of Malaysia (PIGGOTT & PIGGOTT 1996, CHRISTENSEN 1997, 2002) and of the Philippines (QUISUMBING 1951), those of *Botrychium lanuginosum* Wall. ex Hook. & Greville as vegetable in Nepal (MANANDHAR 2002). During a pilot study undertaken in Namibia it has been reported that *O. polyphyllum* may be used as famine food when few other plants are available, but the species is not popular or well-known (LARSEN 2001).

ADDER'S-TONGUE FERN EDIBILITY

Among the different plants mentioned as alternative foods by Ü peasants a fifthteen or so potherbs were listed (Table 1).

Table 1. Wild edible potherbs of South-Central Tibet according to Ü (after Malaisse *et al.* 2003, modified)

Family	Species	Voucher (L & M)	Organe	Use	Translitteration of Tibetan names (Wylie 1959)	Transcription of Tibetan names (Tournadre & Dorje 2003)
Apiaceae	cf. *Angelica* sp.	342, 443	young upper part	green vegetable		cha
Apiaceae	*Carum carvi* L.	278, 435	tender leaf and shoots	green vegetable		koniu, toniu
Asteraceae	*Sonchus brachyotus* DC.	535	young leaf	green vegetable	rgya-khur nag-po	gyakhur nagpo, anggum, umguma
Asteraceae	*Taraxacum* sp. 1	277	young leaf	soup	khurmong	khumo, mo, yo
Asteraceae	*Taraxacum tibeticum* Hand.-Mzt.	499	young leaf	soup	khurmong	ngo, mokhumo
Chenopodiaceae	*Chenopodium album* L.	452	leaf limb	soup	sne'u	neu (lego, nubre)
Malvaceae	*Malva verticillata* L.	357, 449	leaf limb	soup	lcam-pa	champa
Ophioglossaceae	*Ophioglossum polyphyllum* A. Braun in Seub.	371	frond	soup		tuchung
Plantaginaceae	*Plantago depressa* Wild.	450	young leaf	soup	tha-ram	tharam (alathabo, popkera)
Polygonaceae	*Fagopyrum tataricum* (L.) Gaertn.	355, 451,	leaf limb	soup	bra-rgod	tragö, koyowa, torcherawa, pagö
Scrophulariaceae	*Lancea tibetica* Hook. f. & Thoms.	500	leaf	green vegetable	pa-yag-pa	didigonggu, payagpa
Urticaceae	*Urtica dioica* L.	483	young leaf	vegetable, soup	za-po	sapo, suptuk
Urticaceae	*Urtica hyperborea* Jacq. ex Wedd.		young leaf	vegetable, soup	za-po	sapo, suptuk
Urticaceae	*Urtica tibetica* W. T. Wang	514	young leaf	vegetable, soup	za-po	sapo, suptuk
Urticaceae	*Urtica triangularis* Hand.-Mazz.	504	young leaf	vegetable, soup	za-po	sapo, suptuk

Reference collection (L=Leteinturier, M=Malaisse) deposited at Belgium National Botanic Garden [BR].

Tuchung, the Tibetan name of the adder's-tongue fern *Ophioglossum polyphyllum*, has four times be presented, during the interviews conducted in sixty South Central Tibet villages as a valuable legume entering a local soup. This information was provided by inhabitants of three villages located in the same valley in Kyipa community (Netong county, Lhoca prefecture). Young fronds of tuchung are collected in mid-July. The adder's tong fern produces one or two fronds at a time 5 to 15 cm long. with a fertile spike releasing whitish spores at maturity (PIGGOTT & PIGGOTT 1996). The rhizome is cylindrical, tapering from circa 3 mm in diameter, up to 25 mm long, stipe bases are persistent, sterile lamina elliptic to oblong-lanceolate, up to 45 x 25 mm, the fertile spike is inserted at base of the sterile lamina. It grows in rather open places, in poor soil. Our sample was collected at Lhakang (29°22'49"N, 91°49'43"E); reference voucher specimen (LETEINTURIER & MALAISSE 371) is deposited at the Belgium National Botanical Garden (BR according to HOLMGREN et *al*. 1990) at Meise.

Determination up to species level in *Ophioglossum* L. genus appears as a difficult task, as already commented on elsewhere (BURROWS 1996, 1997). Nevertheless, our material matches perfectly with reference material of *Ophioglossum polyphyllum* A. Braun in Seub.

Tuchung is a wanted potherb in this area, being even considered as the best potherb available, before "sapo" (*Urtica* spp.). It is eaten in summer but also dried and stored for further consumption in winter.

NUTRITONAL VALUE OF *Ophioglossum polyphyllum*

Chemical analysis

The fresh plant material (edible part) has been dried on sun. Dry matter was determined from grinded material after drying until constant weight at 110°C. The majority of the major and minor mineral elements have been determined by Atomic absorption spectrometry after acid digestion, excepted SiO2 and chloride which were measured by colorimetry and thiocyanate titration respectively. Fluorides have been determined after sodium hydroxide fusion with a selective ion electrode.

Nitrogen (N) content was measured according to Kjeldahl using Tecator System 20 digestor and auto 1030 Kjeltec apparatus. Amino acid composition was established according to MOORE et *al*. (1958) on an Alpha plus Pharmacia LKB system. Most amino acids were determined after hydrolysis with 6N hydrochloric acid with 0.1% phenol at 110°C for 24 hours under nitrogen. To avoid the loss of cystine and methionine during acid hydrolysis, performic acid oxidation of cystine to cysteic acid and methionine to methionine sulphone was first carried out according to LEWIS (1966).

The protein quality of *O. polyphyllum* was evaluated by calculating the chemical score (or amino acid score). It characterises the balance of essential amino acid and is a useful guide to appreciate protein quality in food (EGGUM 1987). The chemical score is the analytically determined level of the first-limiting amino acid expressed as a percentage of the level of the same amino acid recommended in the Provisional Reference Pattern.

$$\text{Amino acid score} = \frac{\text{FLAAP (mg/g N)}}{\text{FLAAR (mg/g N)}} \times 100$$

FLAAP: amount of the first-limiting amino acid present
FLAAR: amount of the first-limiting amino acid recommended

In the present study, we used the FAO/WHO (1990) Provisional Reference Pattern. Chemical index allowing comparisons with other vegetal protein sources have been established according to FAO/WHO (1989).

After grinding, total lipids were extracted with 2: 1 v/v chloroform - methanol mixture (FOLCH et *al*. 1957). The extracts were concentrated under reduced pressure at 35°C and weighted. Fatty acid methyl esters (FAME) were prepared from crude lipids by boron-trifluoride catalysed transesterification according to AOCS (1998) method and analysed by gas chromatography on a Agilent HP6890 apparatus fitted with a cold-on column injector and a FID detector maintained at 250°C. The operating conditions were fixed as follows: 25m x 0.25 mm CPWAX-FFAP 58 CB Varian column (df = 0.2 μm); temperature programme : from 55°C to 150°C at 30°C.min^{-1} and from 150°C to 230°C at 5°C.min^{-1}. Helium at 1 ml.min^{-1} was used as carrier gas. FAME were identified on the basis of their retention data and by co-injection of pure references. The identifications were confirmed by gas chromatography coupled to mass spectrometry (GCMS) on a Hewlett-Packard HP5971 Mass spectrometer coupled to a HP 5890 Series II gas chromatograph. The chromatographic conditions were the

Table 2.- Mineral composition of Opioglossum polyphyllum (sample FM 15846)

DM (%)	93.24	F (mg/kg DM)	3.9
N_{kjel} (% DM)	3.697	Pb (mg/kg DM)	4.7
P (% DM)	4,9013	Cd (mg/kg DM)	1.06
K (% DM)	4.1817	Cu (mg/kg DM)	17
Ca (% DM)	0.5537	Ni (mg/kg DM)	52
Mg (% DM)	0.3614	Zn (mg/kg DM)	29
Na (% DM)	0.4616	Cr (mg/kg DM)	5.3
Al (% DM)	0.2295	Hg (mg/kg DM)	<0.01
S (% DM)	0.1142	Fe (mg/kg DM)	888
Cl (% DM)	0.0876	Mn (mg/kg DM)	27
SiO_2 (% DM)	1.07	Co (mg/kg DM)	0.4
Ashes (% DM)	8.21	As (mg/kg DM)	1.11

D.M. = Dry matter

same as above and the MS data were obtained in the EI mode at 70eV (Source T°: 250°C, scanned mass range: 35 to 500 amu). The recorded spectra were compared with those of the WILEY275.L computed database and carefully interpreted.

Results

The mineral composition *O. polyphyllum* shown in Table 2 seems not to present any particularity excepted high proportions of phosphorus and of two heavy metals: lead and cadmium. The analysed plants are also characterised by minute amounts of selenium.

Protein level and amino acid profile compositions are summarised in Table 3. O. *polyphyllum contains* 5.54 g % nitrogen which represent 34.62 % proteins (N x 6.25). Acccording to HOCK-HIN and YEOW (1994), it seems that this protein content could be overestimated. Indeed a survey of 90 plants covering taxonomically diverse groups (Pteridophyta, Gymnospermae and Magniolopsida) has highlighted that the traditional nitrogen-to-protein conversion factor of 6.25 is not convenient for plant leaves. The cited authors suggest the use of a conversion factor of 4.43 in order to have reasonably better estimate of protein content. In such a way, the total protein level of *O. polyphyllum* would be limited to 24.5%. Nevertheless, the plant can be "labelled" as a proteagineous vegetable with a well balanced amino acid composition (Table 3). The chemical score (CS) calculated according to FAO/WHO (1990)

Table 3.- Protein composition of *Opioglossum polyphyllum* (mean of three repetitions)

DM (%)	90.67	FAO/WHO values (1989) Essential Amino-Acids	Chemical scores CS (see text)
N_{kjel} (% DM)	5.54		
Protein (% DM)	24.5		
Amino acids (g/100 g protein)			
Aspartic acid	9.70		
Threonine	4.68	3.4	137.7
Serine	5.54		
Glutamic acid	12.75		
Proline	4.01		
Glycine	5.31		
Alanine	5.77		
Cysteine-S-S-Cysteine	1.33		
Valine	6.04	3.5	172.7
Methionine	1.58		
Methionine + Cys-Cys	2.91	2.5	116.3
Isoleucine	4.70	2.8	168.0
Leucine	7.97	6.6	168.0
Tyrosine	3.72		
Phenylalanine	4.46		
Tyrosine + Phenyalaline	8.18	6.3	129.8
Histidine	2.26	1.9	119.2
Lysine	6.30	5.8	108.7
Arginine	6.26		

The essential amino acids are indicated in bold.

Table 4.- Lipids composition of *Opioglossum polyphyllum* (mean of three repetitions)

Total lipids (%)	2.8
Fatty acid profile	
Myristic (C 14)	tr
Palmitic (C 16)	15.8
Palmitoleic (C 16:1)	1.9
Hexadecadienoic	0.8
Margaric (C 17)	0.2
Stearic (C 18)	3.5
Oleic + isomer* (C 18:1)	9.3
Linoileic (C 18:2)	15.2
6-octadecynoic (C 18:1 triple bound)	17.1
Linolenic (C 18:3)	13.7
Arachidic (C 20)	0.4
Eicosenoic (C 20:1)	1.8
Docosanoic (C 22)	2.1
Docosenoic (C 22:1)	3.5
Lignoceric (C 24)	2.6
Saturated	24.6
Mono-unsaturated	16.5
Poly-unsaturated**	29.7
Acetylenic	17.1

* cis-vaccenic acid (traces)
** ethylenic fatty acids

reference patterns are also provided in Table 3. As shown, excepted for tryptophane (not determined), the CS values determined for the each essential amino-acids in *O. polyphyllum* are higher than the reference suggested by FAO/WHO. The contents of threonine, valine, isoleucine and tyrosine + threonine are remarkably high. It seems therefore that on a nutritional point of view, the protein is of good quality and contributes to supply an amino acid complement in local Tibetan diets.

The total lipid content of *O. polyphyllum* leaves is low and attained only 2.8 ± 0.1 % dry weight (Table 4). The dark green lipid extract with high chlorophyll concentration was further investigated by GLC and GCMS in order to establish the FAME profile. The total ion current recorded by GCMS revealed at least sixteen well-resolved fatty acids. The analyses of the mass spectra of individual FAME recorded in the EI mode and the measurement of their retention times compared with authentic standards led to the identification of different fatty acids ranging from C14 to C24 (Table 4). Beside these common derivatives generally found in edible oils and fats, an unusual acetylenic compound has also been detected. For identification purposes, the recorded MS data were compared with that of references. The molecular ion (M+. = 294) revealed a C18 derivative bearing one triple bound. The fragmentation pattern {caracteristic ions at m/z (relative intensity)}: 279 (2%), 263(11%), 220(10%),154(58%), 122(28%), 94(91%), 80(Base peak), 67(23%) and 55(24%)} led to the unambiguous identification of the 6-octadecynoic acid methyl ester. Providing that the FAME profiles of different *Ophioglossum* are established, the detection of this unusual molecule could be potentially valuable as a characteristic for chemotaxonomic classification within the genus. The relative proportions of saturated / mono-unsaturated / poly-unsaturated fatty acids in *O. polyphyllum* oil are of 24.6% / 16.5% / 29.7% respectively. The oil is also characterised by the occurrence of three superior homologous with long acyl residues (C22 : 2.1%, C22:1 : 3.5% and C24 : 2.0%) and by unidentified molecules in minute amounts representing a total of 10% of the fatty acid profile. Additional highly selective chromatographic fractionations are needed to isolate and identify these minor compounds.

DISCUSSION

The original observations gathered within the general survey revealed :

a. That an adder's-tong fern is consumed by some people from South Central Tibet as a common vegetable;

b. That as regards of the FAO/WHO criteria, *O. polyphyllum* is a proteagineous plant with high levels of essential amino-acids that can contribute to complement the local diets;

c. That the oil content is low and that it could not influence very significantly nor the total daily lipid intake nor the fatty acid balance.

To the authors' knowledge, the physiological role of acetylenic acid is not established, and because such a molecule prevails in *O. polyphyllum* lipids, further investigations have to be undertaken in order to evaluate the nutritional value of the oil as well as the search for antinutritional factors.

REFERENCES

AOCS 1998. Official methods and recommended practises of the American Oil Chemist's Society. 5th edition, 1200 pp.

BARFORD A.S & KVIST L.P. 1996. Comparative ethnobotanical studies of the Amerindian groups in coastal Ecuador. *Biologiske Skrifter* 46, Kongelige Danske Videnskabernes Selskab, Copenhagen, 166 p. ISBN 87-7304-274-9.

BURROWS J.E. 1996. The genus *Ophioglossum* in South-Central Africa. *In* J.M. Camus, M. Gibby & R.J. Johns (Eds.) : Pteridology in Perspective, Royal Botanic Gardens, Kew, 329-336.

BURROWS J.E. 1997. The use of spore morphology as a taxonomic tool in the delimitation of Southern African species of *Ophioglossum* L. (Ophioglossaceae : Pteridophyta). *In* R.J. Johns (ed.) : Holttum Memorial Volume, Royal Botanic Gardens, Kew (U.K.), 43-65

CHERIKOFF V. 1993. The bushfood handbook. How to gather, grow, process & cook Australian wild foods. Bush Tucker Supply Australia Pty Ltd, Boronia Park (Australia), 208 p. ISBN 0-646-15496-6.

CHRISTENSEN H. 1997. Uses of ferns in two indigenous communities in Sarawah, Malaysia. *In* R.J. Johns (ed.) : Holttum Memorial Volume, Royal Botanic Gardens, Kew (U.K.) 177-192.

CHRISTENSEN H. 2002.Ethnobotany of the Iban & the Kelabit. Nepcon, Univ.of Aarhus. ISBN 87-988773-0-5

COPELAND E.B. 1942. Edible ferns. *Amer. Fern.* J. 32: 121-126.

CUYPERS J-B. 1970. *L'alimentation chez les Shi*. Musée royal de l'Afrique centrale, Tervuren (Belgique). Annales, série in 8°, Sciences humaines, 67: 249 p.

EGGUM B.O. 1987. The nutritional quality of protein in legumes and oil crops and the role of antinutritional factors. *In* V. Pattakou (Ed.): Protein evaluation in cereal and legumes. A seminaire in the CEC programme of coordination of agricultural research on plant productivity, held in Thessaloniki, 23-24 October 1985.

FAO/WHO 1989. Report of the joint expert committee on Food additives. WHO Food Additive Series, n°776.

FAO/WHO 1990. Report of the joint expert consultation on protein quality evaluation. FAO, Rome 4-8 December 1989.

HAUBRUGE E., CHASSEUR C., MATHIEU F., BEGAUX F., MALAISSE F., NOLARD N., ZHU D., SUETENS C. & GASPAR C. 2000. La maladie de Kashin-Beck et le milieu rural au Tibet: un problème agri - environnemental. *Cah. Agric*. 9: 117-124.

HOCK-HIN Y. & YEOW-CHIN W. 1994, Leaf protein contents and nitrogen-to-protein factors for 90 plant species. *Food Chem.* 49, 3: 245-250

HOLMGREN P.K., HOLMGREN N.H. & BARNETTE L.C. 1990. *Index Herbariorum. Part I : The Herbaria of the world.* 8th edit., New-York Botanical Garden, New York (U.S.A.) : 693 p.

ISAACS J. 1994. Bush food : *Aboriginal food and herbal medicine.* Lansdowne, Sydney (Australia), 256 p., ISBN 0 7254 0834 0.

JONES D.L. 1987. Encyclopedia of Ferns. An introduction to ferns, their structure, biology, economic importance, cultivation and propagation. Timber Press, Portland (U.S.A.), 433 p. ISBN 0 88192 054 1

LARSEN A. 2001. OMBIDI. A pilot study in Northern Namibia. www.larsen-twins.dk/ombidi/100ombidi.html

LEWIS O.A.M. 1996. A short ion-exchange column method for the estimation of cystine and methionine. *Nature*, London, 209: 1239-1241.

MALAISSE F. 1997. Se nourrir en forêt claire africaine. Approche écologique et nutritionnelle. Presses Agronomiques de Gembloux/C.T.A.,Wageningen, 384 p.

MALAISSE F. 2001. Lessons from the past for a better future : Ethnoecology, a promising link between tradition and science regarding biodiversity management. *In* Science and Tradition: Roots and Wings for Development. ARSOM/UNESCO, Brussels, 89-106.

MALAISSE F., BEGAUX F., CHASSEUR C., DOLKAR P., LETEINTURIER B., LOGNAY G., MATHIEU F., RINCHEN L., WATHELET B. & HAUBRUGE E. 2003. Ethno-ecological approach of Tibet South-Central rural environment knowledge as a support to Kashin-Beck disease's etiology. *Bull. Séanc. Acad. r. sci. Outre-mer* 48 (2002-3) : 319-341.

MANANDHAR N.P. 2002. Plants and people of Nepal. Timber Press, Portland (U.S.A.), 599 p. ISBN 0-88192-527-6

MAY L.W. 1978. The economic uses and associated folklore of ferns and fern allies. *Bot. Rev*. 44, 4: 491-528.

MOORE S., SPACKMAN D. & STEIN W. 1958. Chromatography of amino acids on polystyrene sulfonated resins. *Anal. Chem.* 30: 1185-1200.

NORTON H.H. 1979. Evidence for western brackenfern as a food for aboriginal peoples of western Washington. *Econ. Bot.* 33 : 384-396.

PIGGOTT A.G. & PIGGOTT C.J. 1996. *Ferns of Malaysia in colour*. Tropical Press, Kuala Lumpur, 458 p.

PRYER K.M., SCHNEIDER H., SMITH A.R., CRANFILL R., WOLF P.G., HUNT J.S. & SIPES S.D. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618-622.

QUISUMBING E. 1951. Medicinal plants of the Philippine. Tech. Bul. 16. Philippine Dept.Agric. Nat. Res., Manila.

ROWTHORN C., ANDREW D., HELLANDER P. & LINDENMAYER C. 1999. Malaisie et Singapour. Lonely Planet, Melbourne (Australia), 672 p. ISBN 2-84070-093-X

SHARMA S.D. 2000. Food for life. The Tribune, India. http://www.tribuneindia.com/20000130/spectrum/main8.htm

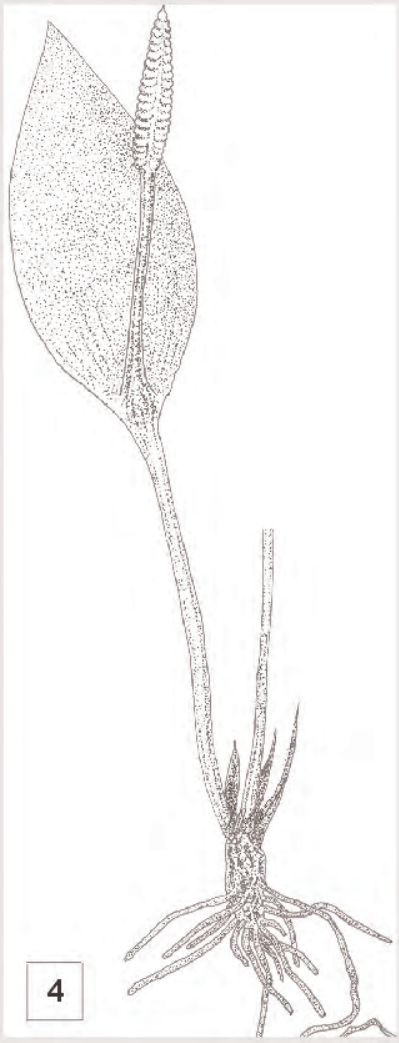
TOURNADRE N. &DORJE S. 2003. Introduction au Tibétain standard (parlé et écrit) suivi d'un appendice consacré au tibétain littéraire classique. Langue et Civilisation. L'Asiathèque, Paris, 543 pp. + viii pl. + 2 cartes.

von ADERKAS P. 1984. Economic history of ostrich fern, *Matteuccia struthiopteris*, the edible fiddlehead. *Econ. Bot*. 38,1: 14-23.

WEE Y.C. 1997. *Ferns of the Tropics*. Times Editions, Singapore, 190 p. ISBN 981 204 793 X.

WYLIE T. 1959. A standard system of Tibetan transcription. *Harvard J. Asiatic Studies* 22: 261-267.

Plate



1a-1b: Habit of *Ophioglossum polyphyllum* in Bangri vegetation near Lhakang (voucher Leteinturier & Malaisse 371)

2 : *Ophioglossum polyphylum*; reference material for analysis (voucher Malaisse 15846)

3 : Material of *Ophioglossum polyphyllum* collected by local inhabitants of Kyipa for consumption

4 : *Ophioglossum polyphyllum*, fertile frond (drawing: C. Van Marsenille)