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
J.-L. WOLFENDER**

UNIVERSITY OF ZIMBABWE PUBLICATIONS

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 J.-L. WOLFENDER

Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne, Switzerland and Department of Pharmacy, University of Zimbabwe, P.O. Box M.P. 167, Harare, Zimbabwe

UNIVERSITY OF ZIMBABWE PUBLICATIONS
1996

First published in 1996 by
University of Zimbabwe Publications
P.O. Box MP 203
Mount Pleasant
Harare
Zimbabwe

ISBN 0-908307-59-4

Cover photos.
African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)

Printed by Mazongororo Paper Converters Pvt. Ltd., Harare

15. Bioactive metabolites from African medicinal plants

C. SPATAFORA AND C. TRINGALI

Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, I-95125 Catania, Italy

Introduction

A peculiarity of African culture is that the near totality of ingredients used for the formulation of medical remedies come from plant sources; when subjected to chemical analysis, these plants frequently afford biologically active substances (Iwu 1994). As part of our search for bioactive natural products, we considered as candidates for chemical analysis some African medicinal plants which are used in Guinea, as in other regions, for the so-called 'cure salée' of cattle. In this prophylaxis, bark, leaves or other parts of the plant are ground with salt and administered to the animals. A number of the plants commonly used for the 'cure salée' are known as folk medicines and have been subjected to chemical analysis; nevertheless, no systematic study aimed at the identification of the active principles involved in this treatment has been carried out to date. Thus, we have collected samples of plants used for the 'cure salée' in the region of Boké (Republic of Guinea) with the aim of subjecting them to a systematic chemical study.

In the isolation of bioactive compounds from natural sources, a variety of criteria can be used to select the more interesting constituents from the complex crude extracts normally obtained by treatment of ground material with solvents. One method is to isolate the main components of the extract, in the assumption that they may be responsible for the activity of the plant. An alternative is to use a biological assay as a guide for fractionation, thus obtaining compounds which are certainly biologically active, at least according to the bioassay used. Tests strictly related to the medical utilization of the plant are generally difficult to perform: indeed, medicinal plants are frequently used for a wide range of different afflictions; moreover, bioassays based on the manipulation of human pathogenic microorganisms require specialized equipment and staff trained in microbiological techniques. To overcome this difficulty, a number of bench bioassays which can be carried out in a chemical laboratory have been developed among them, the brine shrimp test (BST) is convenient for a number of reasons. In this simple test larvae of the small crustacean *Artemia salina* are used as an indicator of toxicity.

This activity can be considered, in a broad sense, as an indicator of bioactivity (Meyer *et al.* 1982). Lethality to *A. salina* larvae has been shown to be related to *in vitro* and *in vivo* antitumor activity; in particular, a correlation with cytotoxic activity against KB and P-388 tumoral cells has been observed (Meyer *et al.* 1982; Ferrigni *et al.* 1984; Anderson *et al.* 1991). In addition, toxicity to *A. salina* larvae has been related to insecticidal activity (Michael *et al.* 1956, Rupprecht *et al.* 1986; Li *et al.* 1990). Brine shrimp test has been used for bioactivity-guided fractionation of plant extracts, and is recommended by National Cancer Institute (Bethesda, Maryland) as an in-house test for the search of promising antitumor compounds (Cragg, G.M., personal communications 1992 and 1995).

For the above cited reasons, we subjected the extracts of the plants collected in Guinea to a preliminary screening based on BST. Bark, leaves or pods of the plants were ground and the powder extracted with ethyl acetate (EtOAc), the residue was subsequently extracted with ethanol (EtOH). A list of the plants subjected to this procedure and the relevant LD_{50} values (μg/ml) are presented in Table 15.1.

Table 15.1. Activity against *A. salina* (BST)[a] of African plant extracts

Plant species	material	LD_{50} (EtOAc)[b]	LD_{50} (EtOH)[b]
Anthocleista djallonensis	bark	>1000	>1000
Bombax constatum	bark	>1000	>1000
Crossopterix febrifuga	bark	>1000	72.8
Dialium guineense	leaves	>1000	140.1
Fagara macrophylla	bark	3.3	15.7
Ficus gnaphalocarpa	bark	>1000	315.1
Lophira lanceolata	bark	>1000	30.7
Markhamia tomentosa	bark	231.1	>1000
Nauclea latifolia	bark	>1000	985.3
Parkia biglobosa	bark	>1000	315.2
Pericopsis laxiflora	bark	190.3	570.1
Piliostigma thonningii	pods	>1000	55.8

[a] Brine shrimp test: mortality of larvae of *Artemia salina*, determined after 24 h exposure. See Meyer (1982) and Tringali (1995) for details of the method.
[b] EtOAc = ethyl acetate extract; EtOH = ethanol extract. Values are measured in μg/ml.

In applications of this test to crude extracts from plant material, an LD_{50} value lower than 1000 μg/ml is considered an indication of activity (Meyer *et al.* 1982), and makes the extract worthy of further analysis. The majority of the plants listed in Table 15.1. showed a notable activity in the ethanolic extract, some of them (*Crossopterix febrifuga, Fagara macrophylla, Lophyra lanceolata, Piliostigma thonningii*) with LD_{50} lower than 100 μg/ml. *Markhamia tomentosa* is active only in the ethyl acetate extract. Interestingly, the two species *Fagara macrophylla*

Engl (Rutaceae; syn. *Zanthoxylum macrophyllum* Miq.) and *Pericopsis laxiflora* Benth (Leguminosae; syn. *Afrormosia laxiflora* Harms) display lethality to *A. salina* larvae in both extracts; in particular, the ethyl acetate extract of *F. macrophylla* is by far the most active one, showing an LD_{50} = 3.3 µg/ml. Confirmation of the biological activity of the EtOAc extract of *F. macrophylla* came from cytotoxicity tests on tumoral cell cultures carried out at the University of Nantes (France): this extract revealed activity against lung tumor cells (IC_{50} = 33.8 µg/ml); further cytotoxicity tests have been carried out at the NCI and a broad spectrum cytotoxicity has been observed (IC_{50} not available). Finally, the EtOAc extract of *F. macrophylla* has also been tested at the NCI against the HIV virus, showing a 'moderate activity' (EC_{50} = 23.4 µg/ml, IC_{50} = 57.1 µg/ml).

In addition to these data, mention should be made of the ethnobotanical importance of plants belonging to the genus *Fagara* (or to the allied *Zanthoxylum*), which are used in folk medicine for the cure of different afflictions (Adesina 1987). *F. macrophylla* is also used as a fish poison and arrow poison (Watt and Breyer-Brandwijk 1962). Analogously, *P. laxiflora* is employed for the treatment of various infirmities, among them fever, pain and snake-bites (Watt and Breyer-Brandwijk 1962; Kerharo 1974).

Both *F. macrophylla* and *P. laxiflora* have been previously subjected to chemical studies. *F. macrophylla* (considered also as a synonym of *Zanthoxylum gillettii* Waterm.) afforded the furoquinoline alkaloid skimmianine (Fish and Waterman 1971a), various benzo[*c*]phenatridine alkaloids (Torto and Mensah 1970; Fish and Waterman 1971b) and neutral compounds like *N*-isobutylamides (Kubo *et al.* 1984; Adesina and Reisch 1988). The only report on bark constituents of *P. laxiflora* concerns the alkaloid *N*-methylcytisine (Fitzgerald *et al.* 1976); flavonoids and further neutral metabolites were obtained from the heartwood (Fitzgerald *et al.* 1976) and from the leaves (Sultana and Ilyas 1987).

None of the above cited studies have been accomplished on the basis of bioassay-guided fractionation and they do not allow the sure identification of the active principles responsible for the BST results; thus, we decided to carry out the chemical analysis of the bark extracts of the plants *F. macrophylla* and *P. laxiflora*, using the BST as a guide for fractionation.

Results and Discussion

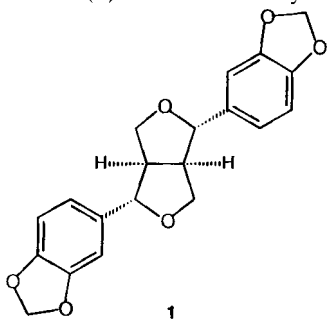
Fagara macrophylla

The ground bark of *F. macrophylla* was extracted with EtOAc and subsequently with EtOH. The less active (LD_{50} = 15.7 µg/ml) ethanolic extract was partitioned between water and ethyl acetate; the BST of these fractions showed that the activity against *A. salina* was retained by the organic phase (LD_{50} = 9.4 µg/ml; aqueous phase, LD_{50} = 246.4 µg/ml). The EtOAc extracts were joined on the basis of their similarity (TLC) and the whole organic extract was

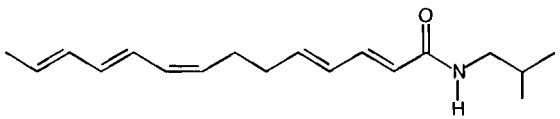
subjected to an activity-guided flash-chromatography on acetyl-polyamide. Fractions showing a similar TLC profile were pooled (A-F) and subjected to the BST. Only subfractions showing LD_{50} lower than 200 µg/ml (B-E) were promoted to further isolation work.

The elucidation of the structures of the metabolites isolated from the active fractions B-E has been based essentially on spectral analysis. The established structures were used for an on-line literature check, which allowed the identification of previously known compounds. When necessary, two-dimensional NMR methods were applied to achieve the complete assignments of ^1H and/or ^{13}C NMR spectra of the purified compounds.

The two major constituents obtained from fraction B (LD_{50} = 3.6 µg/ml) were identified as the lignan (-)-sesamin (**1**) and the *N*-isobutylamide γ-sanshoöl (**2**).



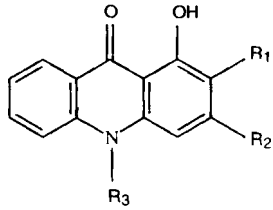
1



2

The ^1H NMR spectrum of γ-sanshoöl (**2**) was previously only partly assigned (Yasuda *et al.* 1981); the complete, revised assignment of this spectrum was an intriguing task, because of the severe overlapping of the low-field signals, and was achieved by the use of advanced homonuclear and heteronuclear 2D-NMR techniques (COSY, TOCSY, NOESY, HMQC and HMBC), performed at 500 MHz. The results of the NOESY experiment, coupled with careful $^3J_{H,H}$ measurements, allowed to confirm the stereochemistry of the double bonds, previously assigned on the basis of ^{13}C chemical shift analysis.

Fractions C (LD_{50} = 3.4 µg/ml) and D (LD_{50} = 21 µg/ml), afforded as main components the known acridone alkaloids 1-hydroxy-3-methoxy-*N*-methyl-acridone (**3**) and arborinine (**4**); analogously, xanthoxoline (**5**) was identified in fraction E (LD_{50} = 54.0 µg/ml). From the same fraction, the previously unreported 1-hydroxy-3-methoxy-acridone (**6**) was isolated.



3: R_1 = H; R_2 = OMe; R_3 = Me

4: R_1 = OMe; R_2 = OMe; R_3 = Me

5: R_1 = OMe; R_2 = OMe; R_3 = H

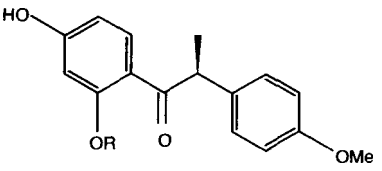
6: R_1 = H; R_2 = OMe; R_3 = H

The structure of **6** was established on the basis of spectral analysis. The molecular formula $C_{14}H_{11}NO_3$ was determined by MS and NMR data. Further physical measurements (IR, UV, ^1H and ^{13}C NMR) strongly suggested a structural similarity with the co-occurring acridones **3** - **5**. In particular, the analysis of both ^1H and ^{13}C NMR spectra of **6**, showed the presence of only one methoxy group, and the lack of the NMe function; conversely, an NH resonance was discernible in the ^1H NMR spectrum at δ 12.10. In the same spectrum, a typical low-field OH resonance was observed at δ 14.22, attributable to an hydroxyl function involved in an intramolecular hydrogen bond with the carbonyl group, and consequently located in C-1. The methoxy group must be located at C-3, because location at C-2 or C-4 would require *ortho*-coupled signals, while typical *meta*-coupled signals appear at δ 6.14 and δ 6.38 (1 H each, *d*, *J* = 2.0 Hz). These assignments were confirmed by ^{13}C NMR chemical shift calculations.

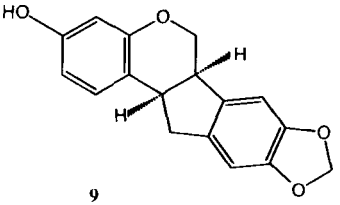
Complete ^1H and ^{13}C NMR assignments were achieved for compound **6**; analogously, the NMR data available from the literature for metabolites **3** - **5** (Fish and Waterman 1971; Bergenthal *et al.* 1979; Reisch *et al.* 1985) were revised and/or completed. The NMR assignments for compounds **2** - **6** will be reported in an extended paper.

Pericopsis laxiflora

In the present study, only the EtOAc extract of the ground bark of *P. laxiflora* was subjected to chemical analysis, in view of its higher BST activity (LD_{50} = 190.3 μg/ml) with respect to the EtOH extract (LD_{50} = 570.1 μg/ml). Fractionation on acetyl-polyamide, followed by TLC analysis and paralleled by BST, afforded an active subfraction (LD_{50} = 130.3 μg/ml), subjected to further chromatography to give as pure compounds the α-methyldeoxybenzoins *R*-(−)-angolensin (**7**) and *R*-(+)-2-*O*-methyl-angolensin (**8**), and the pterocarpan (−)-maackiain (**9**).



7 R = OH
8 R = OMe



9

The unambiguous identification of compounds **7-9** was based on the concerted use of advanced 2D NMR methods and the preparation of the acetate of **9**. The previously reported ^{1}H NMR data of synthetic (±)-angolensin and (±)-2-*O*-methylangolensin (Jain and Paliwal 1988) were revised and their complete ^{1}H and ^{13}C NMR assignments were determined (Tringali 1995). The identity of **9** with the earlier isolated (−)-demethylpterocarpin was also proved through physico-chemical analysis of its derivative, 3-acetoxy-demethylpterocarpin.

Biological activity of compounds 1-9 and conclusive remarks.

As expected, compounds **1-9** are active against larvae of *A. salina*; their LD_{50} values are reported in Table 15.2.

Table 15.2. Activity against *A. salina* (BST) for compounds **1-9**

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
LD_{50} [a]	2.4	7.1	11.3	3.4	12.4	35.1	28.1	36.0	89.2

[a] Values are measured in µg/ml.

Referring to *A. salina* larvae, compounds **1** and **4** are strongly active; compounds **6** - **9** display a mild activity. Nevertheless, in considering these data it should be remembered that there is no direct quantitative relationship between the BST results and related activities like cytotoxicity against tumoral cells (Solis *et*

al. 1993). With reference to the metabolites obtained from the bark of *F. macrophylla*, mention should be made of the fact that only compound **1** displays an LD_{50} value lower than that determined for the crude EtOAc extract: this suggests that compounds **1** - **6** could act sinergistically.

Interestingly, sesamin (**1**) is known as an insecticidal synergist (MacRae and Towers 1984) and inhibits the growth of *Bombix mori* (Kamikado *et al.* 1975); γ-sanshoöl (**2**) is strictly related to molluscicidal and insecticidal *N*-isobutylamides (Kubo *et al.* 1984); metabolites **3** - **6** belong to the class of acridone alkaloids, an important group of bioactive compounds (Michael 1995, Takemura *et al.* 1995), including antitumor substances (Su and Watanabe 1993); arborinine (**4**) is known for its antispasmogenic activity (Minker *et al.* 1979). No biological data have been previously reported for angolensin **7** and its derivative **8**; the same is true for compound **9**; however, this latter compound is related to bioactive pterocarpans, displaying interesting biological properties, *e.g.* antimicrobial (Kamat *et al* 1981; Mitscher *et al* 1988; Taniguchi and Kubo 1993), antifungal (Perrin *et al* 1972), anti-HIV (Engler *et al* 1993) and cytotoxic (Dagne *et al* 1993) activity.

The above results confirm the reliability of the BST as a guide for fractionation of plant extracts in the search for bioactive compounds. Taking into account the activity against *A. salina* and the above cited biological data, compounds **1** - **6** are currently under investigation for their possible action against insects, HIV virus and tumoral cell cultures.

On the basis of the above discussed data, one may reasonably presume that metabolites **1** - **9** could act as defense substances for *F. macrophylla* and *P. laxiflora* and are probably useful components of the traditional preparations obtained from the bark of these plants.

Acknowledgments

The authors are grateful to Dr. G.M. Cragg and Dr. A.B. Mauger and his staff at the National Cancer Institute, NCI (Bethesda, Maryland), for the human cancer cell line *in vitro* test panel and for the *in vitro* anti-HIV test. Thanks are also due to Prof. J.F. Verbist and Dr. C. Roussakis, SMAB, Université de Nantes (France) for the cytotoxicity test against lung tumoral cells. Acknowledgments are also due to Dr. M. Girelli, Zooconsult Srl (Roma, Italy) for the gift of the plant material, and to Dr. E. Timmermans and Mr. K. Camara (Boké, Republic of Guinea) for the collection and preparation of this material. The authors also acknowledge Prof. N. Longhitano and Dr. E. Cirino, Dipartimento di Botanica, Università degli Studi (Catania, Italy) for the identification of the plants. The work described here was financially supported by the Consiglio Nazionale delle Ricerche (Roma, Italy) and by the Ministero della Pubblica Istruzione, Università e Ricerca Scientifica e Tecnologica (Roma, Italy).

References

Adesina, S.K. (1987). Further new constituents of *Zanthoxylum leprieurii*. *Fitoterapia* **58**, 123-126.

Anderson, J.E., Goetz, C.M., McLaughlin, J.L., and Suffness M. (1991). A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. *Phytochemical Analysis* **2**, 107-111.

Bergenthal, D., Mester, I., Rozsa, Z., and Reisch, J. (1979). ^{13}C-NMR-Spektren einiger Acridon-Alkaloide. *Phytochemistry* **18**, 161-163.

Dagne, E., Gunatilaka, A.A.L., Kingston, D.G.I., Alemu, M., Hofmann G., and Johnson, R.K. (1993). Two bioactive pterocarpans from *Erythrina burana*. *Journal of Natural Products* **56**, 1831-1834.

Engler, T.A., Lynch, Jr., K.O., Reddy, J.P., and Gregory, G.S. (1993). Synthetic pterocarpans with anti-HIV activity. *Bioorganic Medicinal Chemistry Letters* **3**, 1229-1232.

Ferrigni, N.R., McLaughlin, J.L., Powell, R.G., and Smith, C.R. (1984). Use of potato disc and brine shrimp bioassays to detect activity and isolate piceatannol as the antileukemic principle from the seeds of *Euphorbia lagascae*. *Journal of Natural Products* **47**, 347-352.

Fish, F. and Waterman, P.G. (1971a). A note on the chloroform-soluble alkaloids of *Fagara macrophylla*. *Journal of Pharmacy and Pharmacology* **23**, 67.

Fish, F. and Waterman, P.G. (1971b). Chloroform-soluble alkaloids of *Fagara leprieurii*. *Phytochemistry* **10**, 3322-3324.

Fitzgerald, M.A., Gunning, P.J.M., and Donnelly, D.M.X. (1976). Phytochemical examination of *Pericopsis* species. *Journal of the Chemical Society, Perkin Transactions I*, 186-191.

Iwu, M.M. (1994). African medicinal plants in the search for new drugs based on ethnobotanical leads. In *Ethnobotany and the search for new drugs*, Ciba Foundation Symposium 185, pp. 116-126. John Wiley & Sons, New York.

Jain, A.C. and Paliwal P. (1988). A facile synthesis of α-methyldesoxybenzoins including racemates of natural angolensin, 2-O-methylangolensin and 4-O-methylangolensin. *Indian Journal of Chemistry* **27B**, 985-988.

Kamat, V.S., Chuo, F.Y., Kubo, I., and Nakanishi, K. (1981). Antimicrobial agents from an East African medicinal plant *Erythrina abyssinica*. *Heterocycles* **15**, 1163-1170.

Kamikado, T., Chang, C.F., Murakoshi, S., Sakurai, A., and Tamura, S. (1975). Isolation and structure elucidation of growth inhibitors on silkworm larvae from *Magnolia kobus* DC. *Agricultural and Biological Chemistry* **39**, 833-836.

Kerharo, J. (1974). In La Pharmacopée Sénégalaise Traditionelle (Plantes Médicinales et Toxiques), pp 441-443. Editions Vigot Frères, Paris.

Kubo, I., Matsumoto, T., Klocke, J.A., and Kamikawa, T. (1984). Molluscicidal and insecticidal activities of isobutylamides isolated from *Fagara macrophylla*. *Experientia* **40**, 340-341.

Li, X.H., Hui, H.; Rupprecht, J.K., Liu, M., Wood, K.V., Smith, D.L., Chang, C.J., and McLaughlin, J.L. (1990). Bullatacin, bullatacinone, and squamone, a new bioactive acetogenin, from the bark of *Annona squamosa*. *Journal of Natural Products* **53**, 81-86.

MacRae, W.D and Towers, G.H.N. (1984). Biological activities of lignans. *Phytochemistry* **23**, 1207-1220.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* **45**, 31.

Michael, A.S., Thompson, C.G., and Abramovitz, M. (1956). *Artemia salina* as a test organism for bioassay. *Science* **123**, 464.

Michael, J.P. (1995). Quinoline, quinazoline and acridone alkaloids. *Natural Products Report* **12**, 77-89.

Minker, E., Bartha, C., Rozsa, Z., and Szendrei, K. (1979). Antispasmogenic effect of Rutamarin and arborinine on isolated smooth muscle organs. *Planta Medica* **37**, 156-160.

Perrin, D.R., Whittle, C.P., and Batterham, T.J. (1972). The structure of phaseollidin. *Tetrahedron Letters* **17**, 1673-1676.

Reisch, J., Adesina, S.K., and Bergenthal, D. (1985). Constituents of *Zanthoxylum leprieurii* fruit pericarps. *Pharmazie* **40**, 811-812.

Rupprecht, J.K., Chang, C., Cassady, J.M., McLaughlin, J.L., Mikolajczack, K.L., and Weisleder, D. (1986). Asimicin, a new cytotoxic and pesticidal acetogenin from the pawpaw, *Asimina triloba* (Annonaceae). *Heterocycles* **24**, 1197-1201.

Su, T.L. and Watanabe, K.A. (1993). Anticancer Acridone Alkaloids, in *Studies in Natural Products Chemistry* **13**, Atta-ur-Rahman Ed., Elsevier London, 347-382.

Sultana, S. and Ilyas, M. (1987). A new isoflavone from leaves of *Afrormosia laxiflora* Harms. *Indian Journal of Chemistry* **26B**, 799.

Taniguchi, M. and Kubo, I,. (1993). Ethnobotanical drug discovery based on medicine men's trial in the African savanna: Screening of East African plants for antimicrobial activity II *Journal of Natural Products* **56**, 1539-1546.

Torto, F.G. and Mensah, I.A. (1970). Alkaloids of *Fagara macrophylla*. *Phytochemistry* **9**, 911-914.

Tringali, C. (1995). Identification of bioactive metabolites from the bark of *Pericopsis* (*Afrormosia*) *laxiflora*. *Phytochemical Analysis* **6**, 289-291.

Watt, J.M. and Breyer-Brandwijk, M.G. (1962) In *The Medicinal and Poisonus Plants of Southern and Eastern Africa*, p. 730. E. & S. Livingstone Ltd., Edinburgh.

Yasuda, I., Takeya, K., and Itokawa, H. (1981). Two new pungent principles isolated from the pericarps of *Zanthoxylum ailanthoides*. *Chemical and Pharmaceutical Bulletin* **29**, 1791-1793.