Pharmacognosy Research [Phcog Res.]
Vol 1, Issue 4, Jul-Aug, 2009 Page 197-201
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 22 April, 2009
Modified: 02 June, 2009
Accepted: 02 June, 2009

PHCOG RES.: Research Article

GC-MS Analysis of Terpenes from *Ficus mucuso*

Djemgou Pierre C[a], Ngandeu François[a], Hegazy Mohamed-Elamir F[b*], Nkanwen Eric Rene[a], Neguim Gilles[c], Chosson Elisabeth[d], Verité Philippe[d], Tane Pierre[a]

[a]*Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon.*
[b]*Chemistry of Medicinal Plants Department, National Research Center, Giza 12622, Egypt.*
[c]*Department of Organic Chemistry, Faculty of Science, university of Yaoundé, Box 812 Yaoundé;-Cameroon.*
[d]*Faculty of Pharmacy, University of Rouen, 22 Boulevard Gambetta, Rouen Cedex, France.*

** Telephone 002-120073557; fax 002-33370931; e-mail elamir77@yahoo.com*

ABSTRACT

Fractionation of the CH_2Cl_2/MeOH extract of the stem bark of *Ficus mucuso*, a western Cameroonian plant species lead to five main fractions from which eleven terpenes previously reported from other species of the genus were identified. These were based on GC-MS analysis and comparison with the Willey database.

Keywords *:* Moraceae, *Ficus mucuso,* Triterpene, GC-MS

INTRODUCTION

Ficus (Moraceae) is a genus that about 150 plant species distributed worldwide (1). Most of these are very high trees, meanwhile shrubs and rarely herbs often with milky juice are also encountered (1). *Ficus mucuso* is a tree which can reach 30 m height and 2 m of diameter (1, 2). Leaves are alternate, or opposite. Flowers much reduced are unisexual. Stamens are opposite to the sepals. Seeds possess endosperm sometime with curved embryo. The wood is white or brown; the stem bark is red or yellow and can reach 3 cm of thickness (1, 2). It usually grows on mountain and stands as a single tree in farmland. The flowers occurred in June and the fruits become mature in August (1, 2). The Ivory Coast species did not showed any antimalarial property against *Plasmodium falciparum* (3). Locally called Kekah in Fondjomekwet, the leaves are used against skin diseases, fruits are eaten by monkey and the plant is used as fertilizer. Natural acetylated terpenoids are a typical kind of compounds widely isolated from this genus and may constitute its chemotaxonomic marker (4-7). Previous phytochemical investigation reported triterpenes (4-7), flavonoids (8), chromones (9) and alkaloids (10) as chemical constituents of this genus. Many species of the genus *Ficus* were reported to exhibit significant pharmacological properties including: antimicrobial (8), antidiabetic (11) and anticancer (12). A recent phytochemicl investigation of this plant species reported the isolation of Isoderrone, a chromene derivative (12a). In continuation for our investigation of Cameroonian medicinal plant species (13), we focused on the CH_2Cl_2/MeOH (1/1) extract of the stem bark of *Ficus mucuso* Fikhalo and we reported here in its fractionation and identification of eleven terpenoic derivatives from the extract including three steroids and eight pentacyclic triterpene, furthermore mass fragmentation of triterpenes were widely discussed by Budzikiewicz et al. (1963) (14). Structures were identified from GC-MS analysis and comparison with the Willey database.

MATERIALS AND METHODS

General

The solutions prepared were analysed by GC-EIMS (Masslab Trio 1000) in split mode, the chromatographic conditions being the following: Column DB5-MS, 60 m, 0.25 mm internal diameter, 0.25µm film thickness; injector 325° C; initial oven temperature, 100° C (5 mn), 10° C/mn rate up to 240° C, 2° C/mn rate up to 300° C, 1° C rate up to 340° C.

EIMS conditions: source 200° C ionisation potential, 70 eV. Identification of compounds was made trough their EIMS spectra and comparison with Willey database as far as the data already reported from the genus. The compounds could not be further purified since they were already identified with highest level of similarities.

Thin layer chromatography was performed on Merck precoated silica gel plates and the solvent system was hexane- ethyl acetate or CH_2Cl_2-MeOH. UV detection at 254 and 366 nm was used. Column chromatography was carried out using Merck Silica gel 60 (70 - 230 mesh) as adsorbent.

Plant material

The stem bark of *Ficus mucuso* were collected at Fondjomekwet, Upper-Nkam division, Western Region, Cameroon in July 2005. Plant identification was performed by Dr Onana, National Herbarium, Yaoundé;-Cameroon, where a voucher specimen was deposited.

Extraction and fractionation

4 kg of fresh material was cut into pieces, dried and ground to afford 2.5 kg of powder. The latter was extracted in closed glassware at room temperature with 11L of CH_2Cl_2/MeOH (1:1) mixture. Concentration in vacuum afforded 160 g of extract. Part of the latter (50 g) was defatted to give 17 g of fat and 25 g of organic extract. Fractionation on si-gel CC eluted with Hex-CH_2Cl_2 (1:0; 1:1; 0:1) and CH_2Cl_2-MeOH (9.5-0.5; 9:1; 8:2; 0:1). On the basis of their TLC profile, fractions were combined into five major one (A, B, C, D, E). GC-MS analysis revealed the following results: fraction A is a mixture of 1 and 2 (167 mg); fraction B a mixture of 3, 4 and 5 (315 mg); fraction C a mixture of 6, 7 and 8 (800 mg). Fraction D a mixture of compounds 9, 10 and 11 (372 mg).

RESULTS AND DISCUSSION

Fractionation of the CH_2Cl_2-MeOH (1:1) extract of the stem bark of *F. mucuso* on silica gel and Sephadex LH-20 column led to the identification of eleven compounds. This report is the first chemical investigation on this plant species and could constitute a basic tool for future investigation of the genus. Fraction A was obtained as red gum; GC-MS analysis reveals the presence of two compounds which peak were observed at 41.408 and 43.267 mn of retention respectively, suggesting the sample to be a 2 compounds mixture. Further analysis of the compound at 41.408 mn reveals the molecular ion peak at m/z 468 compatible to the molecular formula $C_{32}H_{52}O_2$; a close fragment was found at m/z 453 $[M\text{-}CH_3]^+$. The major peak appeared at m/z 218 in addition to other peak which could be found at m/z 203 or 189. Comparison of these data with the Willey database reveals 98% of similarities with β-amyrin acetate, to which the compound was identified; β-amyrin acetate, (1) is a common triterpene previously isolated from *Ficus hirta* (15).

GC-MS of the compound at 43.267mn (scan 2741, TIC: PHYOSD), reveals the molecular ion peak at m/z 468 compatible to the molecular formula $C_{32}H_{52}O_2$, a fragment was found at m/z 453 $[M\text{-}CH_3]^+$. The basic peak was found at m/z 218; other fragments could be observed at m/z 203 or 189; this fragmentation is 99% comparable to those of α-amyrin acetate (2), from comparison with the Willey database, thus the compound was identified to the later, previously isolated from *Ficus racemosa* (11).

Fraction B, was obtained as a red gum, its GC-MS analysis reveals a mixture of three peaks at 37.720, 38.549 and 39.221 mn of retention respectively consecutive of three compounds in the sample. Separated GC-MS analysis of these peaks gave the following results: The first compound at 37.720 mn afforded a molecular ion peak at m/z 426, compatible to the molecular formula $C_{30}H_{50}O$; a fragment was found at m/z 411 $[M\text{-}CH_3]^+$. The basic peak appeared at m/z 218. Other remarkable peaks could be observed at m/z 203 or 189. Comparison of these data to those from Willey database reveals the compound in hand to be 99% identical to β-amyrin, (3) an oleanane type triterpene, previously isolated from *Ficus cordata* (16). Analysis of the second compound at 38.549 mn afforded a molecular ion peak at m/z 424, compatible to the molecular formula $C_{30}H_{48}O$; an interesting fragment was found at m/z 409 $[M\text{-}CH_3]^+$. The most intense peak was found at m/z 218. Further peaks were observed at m/z 205 and 189. Comparison of these data to those from the Willey database reveals the compound to be 99% identical to 3-keto-urs-12-ene, (4) an oleanane type triterpene, previously reported from *Ficus microcarpa* (17).

Analysis of the third compound at 39.221 mn afforded a molecular ion peak at m/z 426 compatible with the molecular formula $C_{30}H_{50}O$, a fragment was found at 411

$[M\text{-}CH_3]^+$. The major peak appeared at m/z 218, other peaks could be found at m/z 207 and 189. A parallelism of these data and those from the Willey database, led to the identification of the compound to β-amyrin, (5) an oleanane type triterpene, previously reported from *Ficus cordata* (16).

Fraction C was obtained as a yellow oil mixture. Its analysis on GC-MS reveals three peaks at 36.793, 38.523 and 40.994 mn of retention respectively suggesting the sample to contain three compounds.

Analysis of the compound at 36.793 mn reveals the molecular ion peak which was also the major peak, at m/z 414, compatible to the molecular formula $C_{29}H_{50}O$. Further peaks were observed at m/z 396 $[M\text{-}H_2O]^+$, 381 $[M\text{-}H_2O\text{-}CH_3]^+$. The compound was identified to beta sitosterol (6), previously reported from *Ficus hirta* (18); from comparison of these data to those from the Willey database as 97% similarities were observed.

GC-MS analysis of the compound appearing at 38.523 mn, reveals the major peak which was also the molecular ion one at m/z 412, corresponding to the molecular formula $C_{29}H_{48}O$. Other peaks could be found at m/z 399 $[M\text{-}CH_3]^+$ and 255 characteristic to sterol. Comparison of these data to those from the Willey database reveals 96% of similarity with stigmasterol (7), a common compound to plant species (18), to which the compound was identified.

GC-MS analysis of the compound at 40.994 mn gave the following results: the molecular ion peak appeared at m/z 412, compatible to the molecular formula $C_{29}H_{48}O$. The basic ion peak at m/z 124. Other remarkable peaks were observed at m/z 397 $[M\text{-}CH_3]^+$ and 229. The data were 99% close to those of the Willey database for sitosterone, from this information; the compound was identified to the later, (8) which has been reported from other plants species of the genus (19).

Fraction D was obtained as a yellow gum mixture; its GC-MS analysis reveals three peaks at 38.348, 39.421 and 43.039 mn of retention respectively, suggesting the sample to contain three compounds. Analysis of the compound at 38.348 mn reveals the molecular ion peak at m/z 424; compatible to the molecular formula $C_{30}H_{48}O$, a fragment was found at m/z 409 $[M\text{-}CH_3]^+$. The basic peak was found at m/z 205. Common peak to triterpene

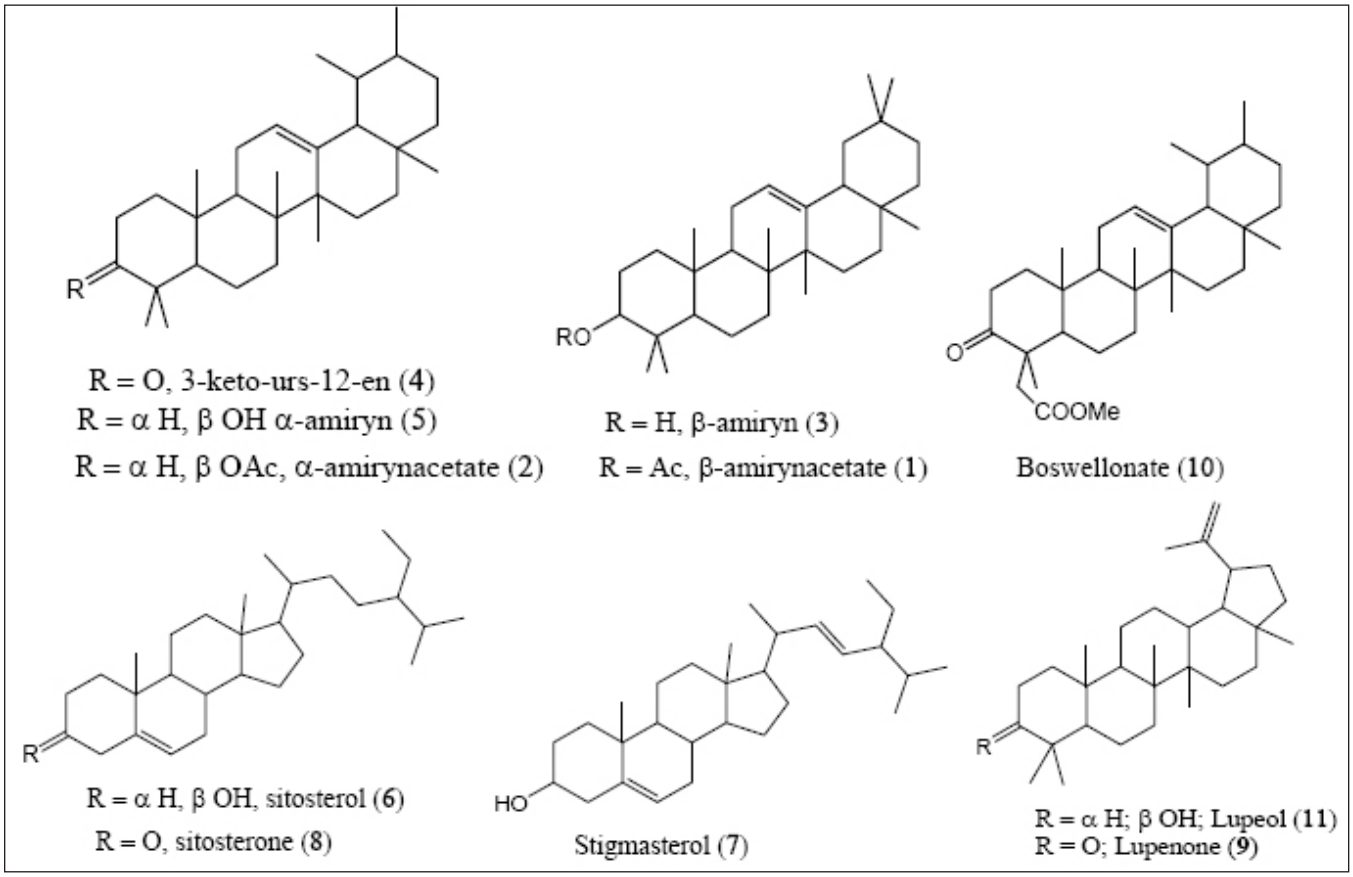

Figure 1. Structures of the identified compounds

were observed at *m/z* 218, 205 and 189. The compounds was identified to lupenone (9) as it showed 99% similarity with the fragmentation of the later from Willey database comparison, this secondary metabolite was previously reported from *Ficus microcarpa* (17).

Analysis of the compound at 39.421 mn afforded the molecular ion peak at *m/z* 468, compatible to the molecular formula $C_{31}H_{48}O_3$; a fragment was found at *m/z* 453 $[M\text{-}CH_3]^+$. The major peak was found at *m/z* 218, other less intense peak were observed at *m/z* 203 or 189, the fragmentation on comparison with Willey database gave 97% of similarities with Boswellonate, (10) to which the compounds was identified (20).

Analysis of the compound at 43.039 mn reveals the molecular ion peak at *m/z* 426; compatible to the molecular formula $C_{30}H_{50}O$, a close fragment was found at *m/z* 411 $[M\text{-}CH_3]^+$. The basic ion peak was found at *m/z* 68. Common peak to triterpene were observed at *m/z* 218, 207 and 189. The compounds was identified to lupeol as it showed 95% similarity with the fragmentation of the later 11, from Willey database comparison, this secondary metabolite has been reported from *Ficus cordata* (16).

ACKNOWLEDGMENT

The authors are grateful to the Service de la Coopé;ration et d'Action Culturelle (SCAC) of the France Foreign Ministry, through its embassy at Yaoundé;, Cameroon for

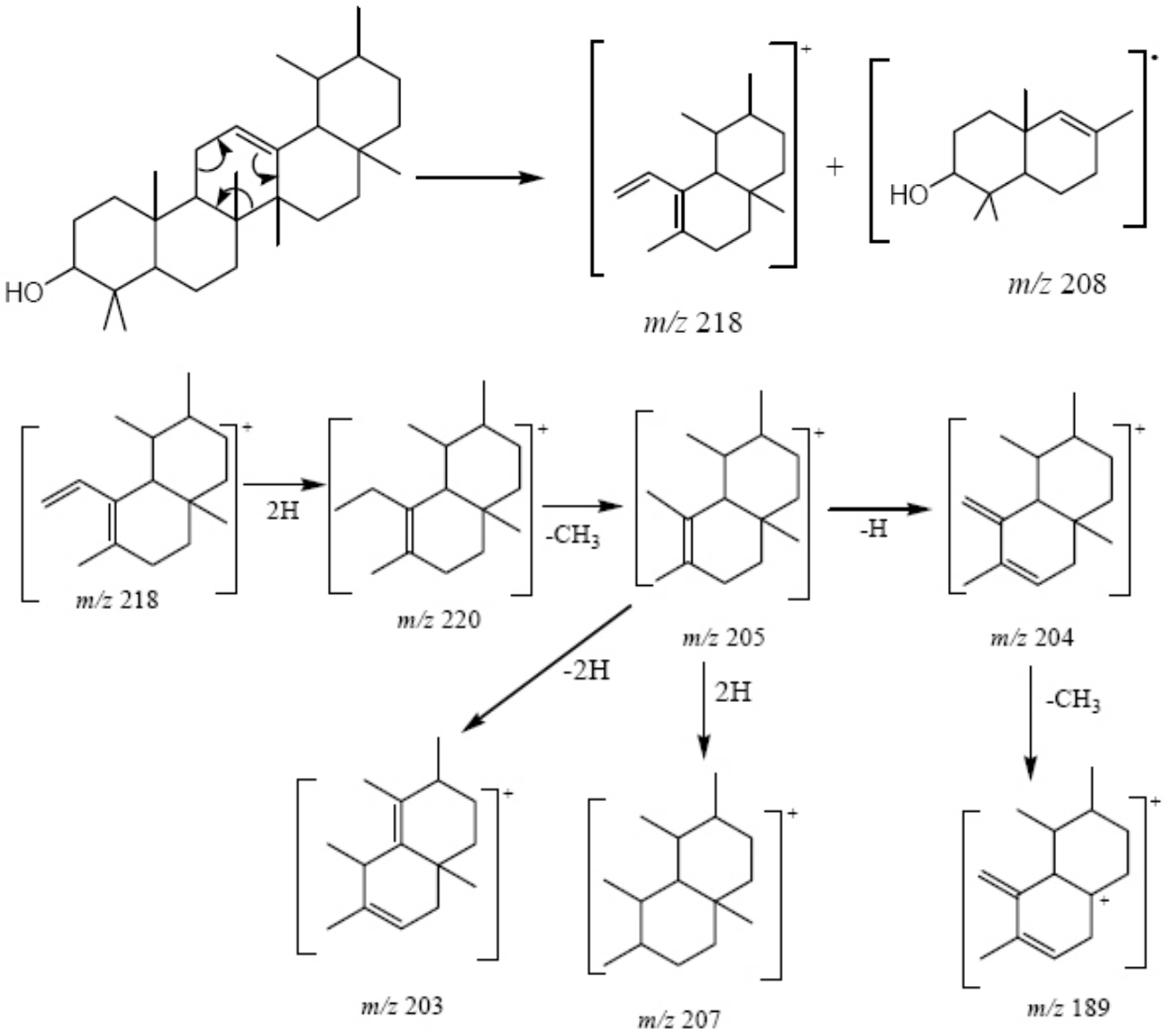

Figure 2. Common Major Fragments of Pintacyclic Triterpene

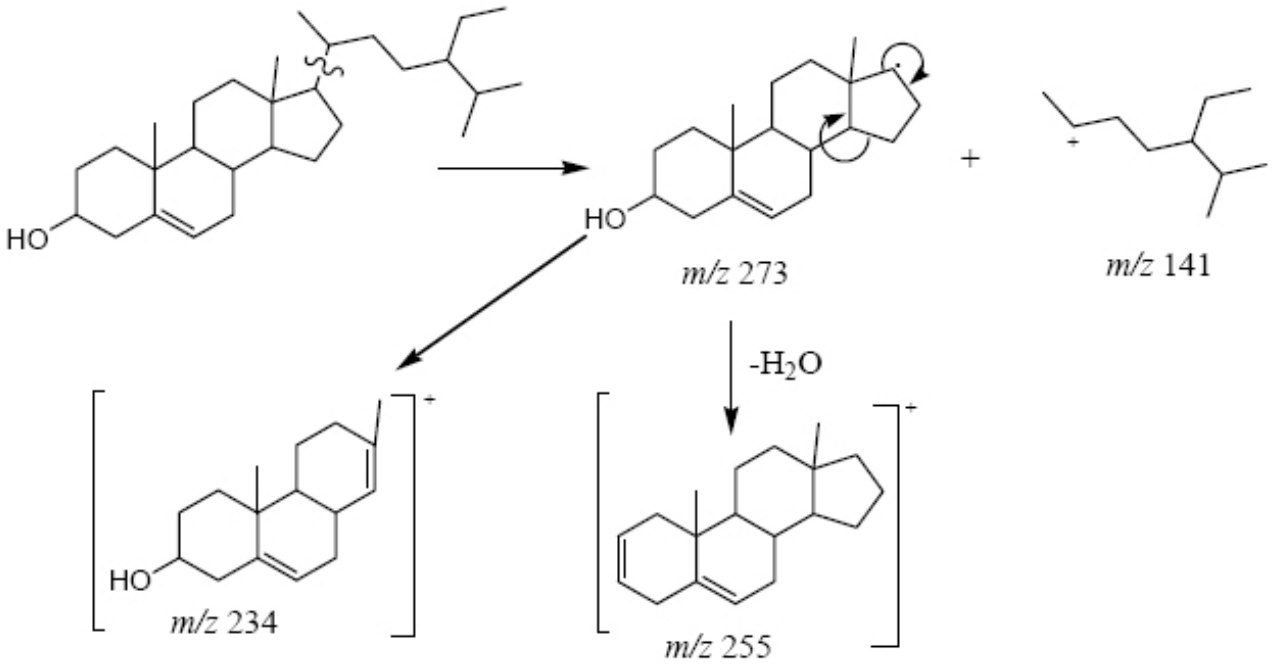

Figure 3. Common Major Fragment for Tetracyclic Triterpenes

financial support of the work; we equally address thanks to Dr Onana for plant identification.

REFERENCES

1. Hutchinson J., Dalziel J.M. and Keay R.W.J., 1958. Flora of West Tropical Africa. second ed Vol 1, p. 600.
2. Letouzey R., 1985. Notice de la Carte Phytogéographique du Cameroun, Imprimerie Jouve, Toulouse, France, p. 82
3. Atindehou Kamanzi K. Schmid C., Brun R., Koné M.W., Traore D., Anti-trypanosomal and Antiplasmodial Activity of Medicinal Plants from Côte d'Ivoire. *Journal of Ethnopharmacology*. **90**, 221–227(2004).
4. Chiang Y.-M., Chang J.-Y., Kuo C.-C., Chang C.-Y., Kuo Y-H., 2005. Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa*. *Phytochemistry* **66**, 495–501.
5. Chiang Y.M. and Kuo Y.H., 2000. Taraxastane type triterpenes from the aerial roots of *Ficus microcarpa*. *J.Nat.Prod.* **63**, 898–901.
6. Chiang Y.M. and Kuo Y.H., 2002. Novel triterpenoids from the aerial roots of *Ficus microcarpa*. *JOC* **67**, 7656–7661.
7. Chiang Y.M. and Kuo Y.H., 2001. New peroxy triterpenes from the aerial roots of *Ficus microcarpa*. *J.Nat.Prod.* 2001. **64**, 436–439.
8. Kuete V., Ngameni B., Simo C.C., Tankeu R.K., Ngadjui B.T., Meyer J.J., Lall N. and Kuiate J.R., 2008. Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa Ficus cordata* (Moraceae). *J. Ethnopharmacology* **120**, 17–24
9. Basudan O.A., Ilyas M., Parveen M., Muhisen H.M., and Kumar R., 2005. A new chromone from *Ficus lyrata*. *J. Asian Nat. Prod. Res.* **7**, 81–85.
10. Damu A.G., Kuo P.C., Shi L.S., Li C.Y., Kuoh C.S., Wu P.L. and Wu T.S., 2005. Phenanthroindolizidine alkaloids from the stems of *Ficus septica*. *J. Nat.Prod.* **68**, 1071–1075
11. Narender T., Khaliq T., Singh A.B., Joshi M.D., Mishra P., Chaturvedi J.P., Srivastava A.K., Maurya R., Agarwal S.C., 2008. Synthesis of alpha-amyrin derivatives and their in vivo antihyperglycemic activity. *Eur J. Med. Chem.* Sep 19
12. Lansky E.P., Paavilainen H.M., Pawlus A.D., Newman R.A., 2008. *Ficus spp.* (fig): ethnobotany and potential as anticancer and anti-inflammatory agents. *J. Ethnopharmacology*. **26**, 195–213
13. Kezetas J.J.B., Ndjakou L.B., Ngouela S., Tsamo E., Sewald N., Neumann B., Stammler H.G., 2007. Isoderrone. *Acta Crystallographica, Section E*: **63**, 523–525
14. Hegazy M-E.F., Ali A.A. Ahmed A.A., Djemgou P.C., Tane P., Ahmed M.M., 2008. A new 14,15-dinorlabdane Glucoside from *Crassocephalum mannii*. *Natural Product Communication*. **3**, 869–872.
15. Budzikiewicz H., Wilson J.M., and Djerassi Carl, 1963. Mass spectrum in structural and stereochemical problems. XXXII, Pintacyclic triterpenes. *Journal of the American Chemical Society*. **85**, 3688–3699.
16. Li C., Bu P.B., Yue D.K., and Sun Y.F., Chemical constituents from roots of *Ficus hirta*. *Zhongguo Zhong Yao Za Zhi*. **31**, 131–3.
17. Poumale Herve M.P., Kengap Rodrigue T., Tchouankeu J.C., Keumedjio F., Laatsch H., Ngadjui B.T., 2008. Pentacyclic triterpenes and other constituents from *Ficus cordata* (Moraceae). *Zeitschrift fuer Naturforschung*. **63**, 1335–1338
18. Yueh-Hsiung Kuo. Lin Huan-You , 2004,. Two novel triterpenes from the leaves of *Ficus microcarpa*. *Helvetica Chimica Acta*. **87**, 1071–1076
19. Chun Li, Pengbin Bu, Dangkun Qiu, Youfu Sun, 2006. Chemical constituents from roots of *Ficus hirta*. *Zhongguo Zhongyao Zazhi*. **31**, 131–133
20. Yongxia Guan, Xiaosheng Yang, Lihua Tong. Bo Yang, Xiaojiang Hao, 2007. Chemical constituents in *Ficus tikoua* of Miao nationality. *Zhongcaoyao*. **38**, 342–344
21. Graham A.G., 1968. The stereochemistry of the boswellic acids. *Phytochemistry*. **7**, 963–73