



Biflavones and triterpenoids isolated from *Ouratea castaneifolia* (DC.) Engl., Ochnaceae

*Luís Adriano S. do Nascimento,¹ Giselle M. S. P. Guilhon,^{*1} Mara S. P. Arruda,¹ Lourivaldo S. Santos,¹ Alberto C. Arruda,¹ Adolfo H. Müller,¹ Milton N. da Silva,¹ Silvane T. Rodrigues,² Mário G. de Carvalho³*

¹Faculdade de Química, Instituto de Ciências Exatas e Naturais, Universidade Federal do Pará, Av. Augusto Corrêa, 1, 66075-110 Belém-PA, Brazil

²Departamento de Botânica, Embrapa Amazônia Oriental, Tv. Enéas Pinheiro s/n, 66077-530 Belém-PA, Brazil

³Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, BR-465, km 7, 23851-970 Seropédica-RJ, Brazil

RESUMO: “Biflavonas e triterpenóides isolados de *Ouratea castaneifolia* (DC.) Engl., Ochnaceae”. O presente trabalho trata da investigação química das folhas e caule da espécie *Ouratea castaneifolia* (DC.) Engl., sobre a qual não há registros de estudos químicos ou farmacológicos anteriores. O estudo fitoquímico clássico dos extratos orgânicos do caule e das folhas de *O. castaneifolia* foi aliado à técnica da cromatografia líquida de alta eficiência (CLAE) e resultou na identificação de dezessete metabólitos: sete triperpenos (friedelina, 3 β -friedelinol, α -amirina, β -amirina, lupeol, taraxerol e germanicol), quatro esteróides (sitosterol, estigmasterol e os glucosídeos sitosteril 3-*O*- β -D-glicopiranosídeo e estigmasteril 3-*O*- β -D-glicopiranosídeo), uma isoflavona (5,7,4'-trimetoxiisoflavona), uma flavona (5,4'-diidroxí-7,3',5'-trimetoxiflavona), quatro biflavonas (amentoflavona, 7,7''-*O*-dimetil-amentoflavona, heveaflavona e tetrametilamentoflavona). A identificação das substâncias foi feita com base na análise de espectros de RMN de ¹H, ¹³C e técnicas bidimensionais. As classes dos metabólitos identificados estão de acordo com aquelas citadas em estudos químicos do gênero *Ouratea*.

Unitermos: *Ouratea castaneifolia*, Ochnaceae, biflavonas, flavonóide, terpenóide.

ABSTRACT: This paper presents the chemical investigation of the leaves and stems of *Ouratea castaneifolia* (DC.) Engl. There are no chemical or pharmacological studies with this species. Classic phytochemical investigation of the organic extracts together with high pressure liquid chromatography (HPLC) procedures lead to the identification of seventeen metabolites: seven triterpenes (friedelin, 3 β -friedelinol, α -amyrin, β -amyrin, lupeol, germanicol and taraxerol), four steroids (sitosterol, stigmaterol and the glycosides sitosteryl 3-*O*- β -D-glucopyranoside and stigmateryl 3-*O*- β -D-glucopyranoside), one isoflavone (5,7,4'-trimethoxyisoflavone), one flavone (5,4'-dihydroxy-7,3',5'-trimethoxyflavone) and four biflavones (amenthoflavone, 7,7''-*O*-dimethylamenthoflavone, heveaflavone and tetramethylamenthoflavone). The structures of the compounds were established by the analysis of ¹H, ¹³C NMR spectra including bidimensional techniques. The classes of the identified metabolites are in agreement with previous studies of the *Ouratea* genus.

Keywords: *Ouratea castaneifolia*, Ochnaceae, biflavones, flavonoid, terpenoid.

INTRODUCTION

Ouratea and other genera of Ochnaceae are a rich source of flavonoids and biflavonoids and according to Suzart et al. (2007), the biflavonoids can be used as chemotaxonomic markers of the genus *Ouratea* as well as of *Luxemburgia* from the same botanic family. Lignans, triterpenes, diterpenes, steroids, monosaccharide, depsides,

triglycerides and chloroisoflavones were also reported from this genus (Velandia et al., 1998a and 1998b; Carvalho et al., 2000; Manga et al., 2001; Mbing et al., 2003a and 2003b; Felício et al., 2004; Estevam et al., 2005). Some biflavonoids, as well as extracts of the *Ouratea* species showed important biological activities, as was previously published (Suzart et al., 2007).

Ouratea castaneifolia (DC.) Engl. Ochnaceae

* E-mail: giselle@ufpa.br; Tel: +559132018099

was collected in Marajó Island in the North of Brazil. This species is known in the Amazon region of Brazil as “farinha-seca”, “mangue-do-mato” or “pau-de-serra” and its wood is used in small constructions and its bark is tonic and adstringent and contains tannins (Le Cointe, 1934). This work describes the phytochemical study of the extracts from the leaves and stems of *O. castaneifolia*.

MATERIAL AND METHODS

General procedures

Melting points are uncorrected. NMR spectra were recorded on a Mercury-300 Varian spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C) using the solvent (DMSO-d_6 , $\text{Me}_2\text{CO-d}_6$, CD_3OD or CDCl_3) and TMS as internal standard. Silica gel (Merck and Vetec 0.05-0.20 mm) or Sephadex LH-20 was used on separations by column chromatography (CC). Silica gel HF (Merck) was used for TLC and revealed by UV (254 and 366 nm), acid solution of ceric sulphate and exposure to iodine vapor. HPLC-UV analyses were performed using a Shimadzu (Shimadzu, Tokyo, Japan) liquid chromatography modular system consisting of two LC-10AD pumps, an UV-Vis Shimadzu SPD-10AV detector, and an LC Workstation Class LC-10 system for data processing. The samples were introduced using an injection valve fitted with 20 μL loop (Rheodyne, California, USA). The mobile phase consisted of water:methanol:acetonitrile (24:40:36, isocratic mode) at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. A C18 column (Gemini, 250 mm x 4.6 mm x 5 μm) fitted with guard column (Gemini, C18, 4 mm x 3 mm x 5 μm) was utilized. UV detection was performed at 330 nm.

Plant material

Leaves and stems of *Ouratea castaneifolia* (DC.) Engl. Ochnaceae were collected in Salvaterra (Marajó Island, State of Pará, Brazil) and identified by one of the

authors (S.T.R.). A voucher specimen (IAN 172171) is deposited at the herbarium of Embrapa-Amazônia Oriental (Belém, Brazil).

Extraction and isolation

Dried and powdered leaves (2500 g) and stems (1880 g) were extracted with *n*-hexane, dichloromethane and methanol by percolation at room temperature. The solutions of leaves (L) and stems (S) were concentrated under vacuum to yield the *n*-hexane (H), dichloromethane (D) and methanolic (M) extracts from the leaves (extract LH: 28.25 g, extract LD: 51.85 g and extract LM: 238.10 g) and stems (extract SH: 3.40 g, extract SD: 2.30 g, extract SM: 32.00 g). Extracts LH (28.25 g), LD (25.00 g) and SD (2.30 g) were fractionated by CC on silica gel using mixtures of hexane with EtOAc and MeOH gradually increasing polarity as elluents. The fractions were purified using similar CC procedures.

The fractions from LH extract eluted with *n*-hexane:EtOAc (6:8) afforded two mixtures of substances, one of compounds **7-11** (102 mg) and the other of **12-13** (11 mg). The fraction from LD extract eluted with EtOAc (100%), named as BF, was analyzed by ^1H NMR spectrum and characteristics signals of a mixture of biflavonoids were identified. After successive CC procedures, including the use of Sephadex LH-20 and methanol as elluent, no separation was achieved; fraction BF was than submitted to HPLC (chromatogram on Figure 1) yielding compounds **14** (4 mg), **15** (4 mg), **16** (4 mg) and **17** (7 mg). Compound **4** was further identified by HPLC from fraction BF. Part of the extract LM (20 g) was suspended on $\text{MeOH:H}_2\text{O}$ (3:1) and successively extracted with CHCl_3 , EtOAc and *n*-BuOH yielding the CHCl_3 , EtOAc and *n*-BuOH phases. During concentration of the CHCl_3 phase from LM extract, a pale yellow solid precipitated and was washed with methanol affording compound **17** (53 mg). The HPLC chromatogram of the CHCl_3 phase from methanolic extract partition was similar to BF fraction.

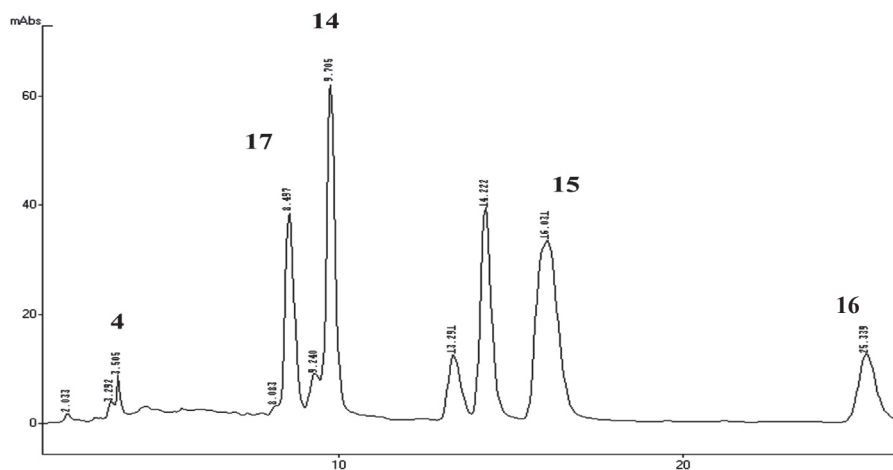


Figure 1. Chromatogram of fraction BF from *Ouratea castaneifolia* (DC.) Engl., Ochnaceae, eluted with $\text{H}_2\text{O}:\text{MeOH}:\text{MeCN}$ (24:40:36). Flow 1 mL/min . UV Detection: $\lambda=330$ nm.

During the concentration of the *n*-hexane solution of the stems (solution SH), a solid material precipitated and was purified by recrystallization with *n*-hexane and EtOAc affording a mixture of substances **1** and **2** (16 mg). Repeated CC procedures of the fractions of extract SD eluted with *n*-hexane:EtOAc (2:8) afforded compound **3** (25 mg); the fraction eluted with *n*-hexane:EtOAc(1:1) yielded compound **4** (5 mg). The fractions from the SM extract (32.00 g) were purified by CC eluting with hexane:EtOAc (25:75) and EtOAc:MeOH (9:1) affording additional quantities of compound **10** (8 mg) and a mixture of compounds **5** and **6** (20 mg).

RESULTS AND DISCUSSION

The chemical study of the stems of *Ouratea castaneifolia* (DC.) Engl. Ochnaceae lead to the identification of the triterpenes friedelin (**1**) (Mahato & Kundu, 1994) and 3 β -friedelinol (**2**) (Salazar et al., 2000), the isoflavone 4',5,7-trimethoxyisoflavone (**3**) (Jha et al., 1980; Wang, 2005) and the flavone 4',5-dihydroxy-3',5',7-trimethoxyflavone (**4**) (Zahir et al., 1996) and two glycosides **5** and **6** identified sitosteryl and stigmasteryl 3-*O*- β -D-glucopyranosides (Chaurasia & Wichth, 1987), respectively.

Table 1. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of **1** in DMSO- d_6 . Chemical shifts are in δ (ppm) and coupling constants (J) in Hz.

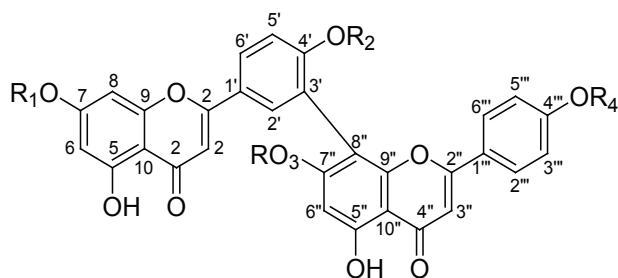
C	HMQC ($^1\text{J}_{\text{CH}}$)		HMBC ($^{2,3}\text{J}_{\text{H-C}}$)
	δ_{CH}	δ_{H} (mult, Hz)	
3	103.3	6.76(s)	C-2, C-4, C-10, C-1'
3''	102.7	6.68(s)	C-2'', C-4'', C-10'', C-1'''
6	98.2	6.31(d, 2.4)	C-7, C-8, C-10
8	92.9	6.66(4, 2.4)	C-6, C-9, C-10
6''	95.6	6.60(s)	C-7'', C-8'', C-10''
2'	131.5	8.09(s, 2.4)	C-2, C-4', C-6', C-8''
5'	128.3	7.26(d, 8.7)	C-1', C-3'
6'	128.2	8.05(dd, 8.7, 2.4)	C-2'
2''', 6'''	128.4	7.65(d, 8.7)	C-3''', 5''', C-2'', C-4'''
3''', 5'''	116.0	6.84(d, 8.7)	C-2''', 6''', C-1'''
MeO-7	56.6	3.82(s)	C-7
MeO-7''	56.2	3.83(s)	C-7''
HO-5	-	12.95(s)	C-6, C-10
HO-5''	-	13.24(s)	C-6'', C-10''

Chemical shift of quaternary carbons					
C	δ_{C}	C	δ_{C}	C	δ_{C}
2	164.2	8''	105.0	10''	104.2
2''	161.4	7	165.3	1'	121.1
4	182.1	7''	162.8	3'	119.8
4''	182.5	9	157.7	4'	159.6
5	161.4	9''	153.7	1''	121.3
5''	161.5	10	104.8	4'''	161.3

^aHomonuclear 2D- ^1H - ^1H -COSY spectra were also used in these assignments.

The triterpenes, α -amyirin (**7**), β -amyirin (**8**), lupeol (**9**), taraxerol (**10**) and germanicol (**11**) (Mahato & Kundu, 1994) and the steroids mixture, sitosterol (**12**) (Nes et al., 1992) and stigmasteryl (**13**) (Forgo & Kövér, 2004), were identified in the extract from the leaves of *O. castaneifolia*. The biflavones **14-17** were also isolated from the leaves of the plant and were identified as 7,7''-di-*O*-methylamenthoflavone (Gu et al., 1990), heveaflavone

(4''', 7, 7''-tri-*O*-methylamenthoflavone) (Carbonezi et al., 2007), 4', 4''', 7, 7'''-tetra-*O*-methylamenthoflavone (Markham et al., 1987) and amenthoflavone (Dora & Edwards, 1991; Markham et al., 1987), respectively.



	R ₁	R ₂	R ₃	R ₄
14	Me	H	Me	H
15	Me	H	H	H
16	Me	Me	Me	Me
17	H	H	H	H

Compound **3** was isolated from *O. hexasperma* (Moreira et al., 1994) and heveaflavone (**15**) from *O. multiflora* (Carbonezi et al., 2007); amenthoflavone (**15**) is common in *Ouratea* (Felicio et al., 2004; Felicio et al., 2001; Velandia et al., 2002). To our knowledge, this is the first occurrence of germanicol (**11**), 4',5'-dihydroxy-3',5',7'-trimethoxyflavone (**4**) and of the biflavone 7,7''-di-*O*-methylamentoflavone (**14**) in the *Ouratea* genus.

All the NMR data of compound **15** (heveaflavone) were in agreement with the literature data (Carbonezi et al., 2007), except for the attribution of the HO-5 and HO-5'' that were changed. In the HMBC of **15**, the signal at δ_{H} 12.95 (OH-5) showed $^2,3\text{JCH}$ correlation with δ_{C} 98.2 (C-6) and the signal at δ_{H} 13.21 (OH-5'') showed $^2,3\text{JCH}$ correlation with δ_{C} 95.6 (C-6'').

The ^{13}C NMR data of compound **14** are being published for the first time. It were made by the 1D (BBD and DEPT) and 2D (HMQC and HMBC) NMR experiments analysis (Table 1). The ^1H NMR spectra [1D and 2D (^1H - ^1H -COSY)] of compound **14** showed signals of two chelated hydroxyls (δ_{H} 12.95 and 13.24), hydrogens of two aromatic methoxyl groups, two hydrogens of ring A (*meta* coupled), one non coupled hydrogen (ring A''), the hydrogens of a 1,3,4-trisubstituted aromatic ring (ring B) and the hydrogens of a *para*-substituted aromatic ring (Ring B''). These ^1H NMR data are in accordance to those published by Gu et al. (1990). The ^{13}C NMR spectrum of compound **14** showed thirty signals, ten signals were attributed to twelve sp^2 CH (including the two signals attributed to C-2''' and C-6''' and to C-3''' and C-5'''), two sp^3 carbons (δ_{CH_3} 56.2 and 56.6), sixteen sp^2 quaternary carbons and two carbonyl groups (δ_{C} 182.1 and 182.5). The structure of **14** was confirmed on the basis of HMBC spectrum (Table 1, Figure 1), which showed heteronuclear long range couplings $^2,3\text{JCH}$ of H-2' with C-8'' and of H-6'' with C-8'. These data together with the absence of

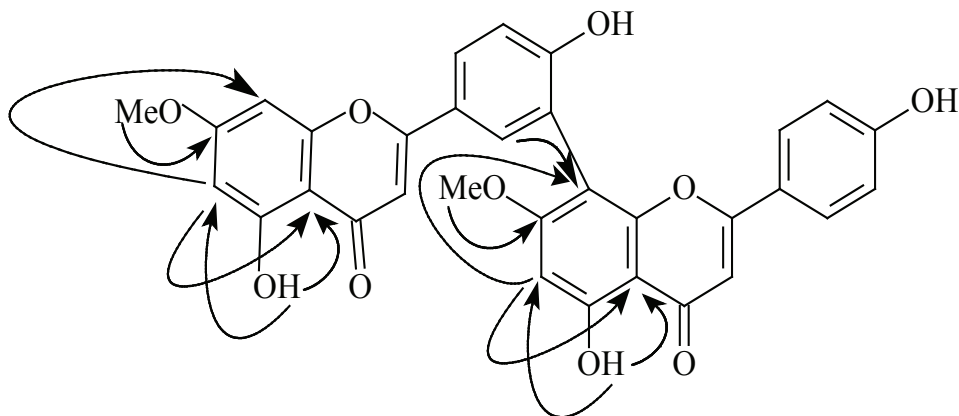


Figure 2. Some $^2,3\text{JCH}$ long-range correlations on the HMBC of compound **14**.

correlations with C-9'', are characteristic of biflavonoids with an amenthoflavone skeleton (Goh et al., 1992). The localization of the methyl groups on compound **14** was deduced by comparison of the chemical shifts on the ^{13}C -NMR spectra with those of amenthoflavone taking in account the protection effect caused by the presence of these groups on the vicinal carbons as described in the literature (Markham et al., 1987). The HMBC experiments also showed correlations of the quaternary carbons C-7'' (δ_{C} 162.8) and C-7' (δ_{C} 165.2) with the hydrogens of the

methoxyl groups at δ_{H} 3.83 (OMe-7'') and 3.82 (OMe-7'), respectively.

ACKNOWLEDGEMENTS

We would like to thank CAPES for the grant (LASN) and CNPq and FUNTEC-SECTAM for the financial support.

REFERENCES

- Carbonezi CA, Hamerski L, Gunatilaka AAL, Cavalheiro A, Castro-Gamboal I, Silva DHS, Furlan M, Young MCM, Lopes MN, Bolzani VS 2007. Bioactive flavone dimers from *Ouratea multiflora*. *Rev Bras Farmacogn* 17: 319-324.
- Carvalho MG de, Carvalho GJA de, Braz-Filho R 2000. Chemical constituents from *Ouratea floribunda*: complete ^1H and ^{13}C NMR assignments of atranorin and its new acetyl derivative. *J Braz Chem Soc* 11: 143-147.
- Chaurasia N, Wichth MJ 1987. Sterols and steryl glycosides from *Urtica dioica*. *J Nat Prod* 50: 881-885.
- Dora G, Edwards JM 1991. Taxonomic status of *Lanaria lanata* and isolation of a novel biflavone. *J Nat Prod* 54: 796-801.
- Estevam CS, Oliveira FM, Conserva LM, Lima LF, Barros ECP, Barros SCP, Rocha EMM, Andrade EHA 2005. Constituintes químicos e avaliação preliminar *in vivo* da atividade antimalária de *Ouratea nitida* Aubl (Ochnaceae). *Rev Bras Farmacogn* 15: 195-198.
- Felicio JD, Rossi MH, Braggio MM, Gonzalez E, Pak A, Cordeiro I, Felicio RC 2004. Chemical constituents from *Ouratea parviflora*. *Biochem Syst Ecol* 32: 79-81.
- Felicio JD, Rossi MH, Park HR, Gonzalez E, Braggio MN, David JN, Cordeiro I 2001. Biflavonoids from *Ouratea multiflora*. *Fitoterapia* 72: 453-455.
- Forgo P, Kövér KE 2004. Gradient enhanced selective experiments in the ^1H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmaterol, a phytosterol derivative. *Steroids* 69: 43-50.
- Goh SH, Jantan I, Waterman PG 1992. Neoflavonoid and biflavonoid constituents of *Calophyllum inophylloide*. *J Nat Prod* 55: 1415-1420.
- Gu YL, Xu YM, Fang SD, He QM 1990. The chemical constituents from *Podocarpus imbricatus*. *Acta Bot Sin* 32: 631-636.
- Jha HC, Zilliken F, Breitmaier E 1980. Carbon-13 NMR shifts and carbon-hydrogen coupling constants of deoxybenzoin and related acetophenones. *Can J Chem* 58: 1211-19.
- Le Cointe P 1934. *Árvores e plantas úteis (indígenas e aclimatadas)*. Série: A Amazônia Brasileira, nº 3. Livraria Clássica, Belém.
- Mahato SB, Kundu AP 1994. ^{13}C NMR spectra of pentacyclic triterpenoids-A compilation and some salient features. *Phytochemistry* 37: 1517-1575.
- Manga SSE, Messanga BB, Sondengam BL 2001. 7,8-Dihydrobenzofuranones from *Ouratea reticulata*. *Fitoterapia* 72: 706-708.
- Markham KR, Sheppard C, Geiger H 1987. ^{13}C NMR Studies of some naturally occurring amentoflavone and hinokiflavone biflavonoids. *Phytochemistry* 26: 3335-3337.
- Mbing JN, Bassomo MY, Pegnyemb DE, Tih RG, Sondengam BL, Blond A, Bodo B 2003a. Constituents of *Ouratea flava*. *Biochem Syst Ecol* 31: 215-217.
- Mbing JN, Pegnyemb DE, Tih RG, Sondengam BL, Blond A, Bodo B 2003b. Two biflavonoids from *Ouratea flava* stem bark. *Phytochemistry* 63: 427-431.
- Moreira IC, Sobrinho DC, Carvalho MG de, Braz-Filho R 1994. Isoflavanone dimers hexaspermone A, B and C from *Ouratea hexasperma*. *Phytochemistry* 35: 1567-1572.
- Nes WD, Norton RA, Benson M 1992. Carbon ^{13}C NMR studies on sitosterol biosynthesized from ^{13}C mevalonates. *Phytochemistry* 31: 805-811.
- Salazar GCM, Silva GDF, Duarte LP, Vieira Filho SA, Lula IS 2000. Two epimeric friedelane triterpenes isolated from *Maytenus truncata* Reiss: ^1H and ^{13}C chemical shift assignments. *Magn Reson Chem* 38: 977-980.
- Suzart LR, Daniel JFS, Carvalho MG, Kaplan MAC 2007. Biodiversidade flavonoídica e aspectos farmacológicos em espécies dos gêneros *Ouratea* e *Luxemburgia* (Ochnaceae). *Quim Nova* 30: 984-987.
- Velandia JR, Carvalho MG de, Braz Filho R 1998a. Ácido ent-16 α ,17-diidroxicauran-19-óico isolado de *Ouratea semiserrata* e os desafios estereoquímicos dos carbonos quirais C-4 e C-16. *Quim Nova* 21: 397-404.
- Velandia JR, Carvalho MG de, Braz Filho R 1998b. Novel trichloro- and tetrachloroisoflavone isolated from *Ouratea semiserrata*. *Nat Prod Lett* 12: 191-198.
- Velandia JR, Carvalho MG de, Braz Filho R, Werle AA 2002. Biflavonoids and a glucopyranoside derivative from *Ouratea semiserrata*. *Phytochem Analysis* 13: 283-292.
- Wang GSF, Jiang Q, Ye YH, Li Y, Tan RX 2005. Genistein derivatives as selective estrogen receptor modulators: Sonochemical synthesis and *in vivo* anti-osteoporotic action. *Bioorgan Med Chem* 13: 4880-4890.
- Zahir A, Jossang A, Bodo B 1996. DNA Topoisomerase I inhibitors: Cytotoxic flavones from *Lethedon tannaensis*. *J Nat Prod* 59: 701-703.