Research Article

Isolation of Sterols, Triterpenoids and a Sesquaterpene from the Leaves of *Ficus nitida* Thunb.

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ABSTRACT

Ficus nitida Thunb., syn. *F. benjamina* (Moraceae), is an evergreen, lacticiferous flowering plant native to south-eastern Asia and Australia. The plant is used to treat influenza, dysentery, malaria, respiratory track diseases, bruises, wound, skin disorders, inflammation, piles, vomiting, leprosy, malaria, nose-diseases, cancer as a general tonic. The dried leaf powder of *F. nitida* was extracted with methanol exhaustively and the combined extracts were concentrated to get a dark brown viscous mass. It was dissolved in minimum amount of methanol, adsorbed on silica gel for column air-dried and chromatographed over a silica gel column packed in petroleum ether. The column was eluted successively with various combinations of petroleum ether, chloroform and methanol in order of increasing polarity to isolate a new sesquaterpene (ficusesquaterpene) along with the known compounds stigmasterol, β-sitosterol, friedelin and β-amyrin. The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords: Ficus nitida, leaves, sterol, triterpenoids, ficusesquaterpene, isolation.

INTRODUCTION

Ficus nitida Thunb., syn. *F. benjamina* (Moraceae), commonly known as weeping fig, benjamin fig or ficus tree, is an evergreen, lacticiferous flowering plant native to eastern Asia and Australia. It is distributed in China, India, Nepal, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Philippines, New Guinea, Australia, Pacific Islands and is the official tree of Bangkok. It is a fast growing shrub or tree, up to 30 m tall, multiple-stemmed , spreading , strangler with multiple aerial stilt roots, stem with white latex, drooping foliage, ripe fruit red –orange, extensive and strong root system. The thick, shiny, alternate, simple, entire, elliptic, two to five-inch-long, evergreen leaves generously clothe the long branches. The branches weep toward the ground forming a canopy so dense that nothing grows beneath them. The axillary, unisexual, monoecious flowers are borne in the fig body. The fruits are fleshy, obovoid or subglobose, turn yellow via orange and dark red [1-2]. The plant is used to treat influenza and dysentery, malaria and respiratory track diseases. The latex mixed with alcohol is used to relieve shocks [2]. The bark of the root, the root itself, and the leaves are boiled in oil and applied on wounds and bruises. The bark juice is utilized in the Philippines to cure liver diseases. The pounded leaves and bark are used as a poultice to alleviate rheumatic pain. The aerial root is taken as a diuretic and to prevent kidney disfunction with mucous, yellow or red urine, abnormal diseases and frequent urination. The plant sap from all plant parts possesses the toxic principles furocoumarins, psoralens, and ficin, which cause minor skin irritation, itching of the eyes, cough and wheezing[3,4]. Its latex and fruit extracts are useful to subside skin disorders, inflammation, piles, vomiting, leprosy, malaria, nose-diseases, cancer and as a general tonic. The plant is also useful as antimicrobial, antinociceptive, antipyretic, hypotensive and anti-dysentery remedy. The leaves and twigs are used as insect repellant[5-7].

The leaves, bark and fruits contain cinnamic acid , lactose , naringenin , quercetin , caffeic acid and stigmasterol[8, 9]. The leaves afforded lutein, β-sitosterol, stigmasterol, chlorophyll a , phytol, (E)-3-alkenoic acid, triglycerides, fatty alcohols and fatty acids[9,10], essential oil [11], isoflavonoids[12] and flavone glycosides[13] . The leaves and barks yielded isoquinoline, indole and quinolizidine type of alkaloids [14]. HPLC analysis indicated the presence of four phenolic compounds (chlorogenic, *p*-coumaric, ferulic and syringic acids) in the roots, three compounds (chlorogenic *p*-coumaric and ferulic acids) in the stem and only one (caffeic acid) in the leaves [16] . This paper describes isolation and characterization of two each of sterols and triterpenoids and a sesquaterpene from the leaves of *F. nitida*.

MATERIALS AND METHODS

General procedures

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on a Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were scanned on Advance DRX Bruker spectrospin 400 and 100 MHz, respectively, instruments (Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were obtained by effecting FAB ionization at 70 eV on a JEOL-JMS DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on a silica gel (60-120 mesh, Qualigen, Mumbai, India) column. TLC was run on silica gel G (Qualigen) coated plates. Spots were visualized by exposing to iodine vapors, UV radiation and spraying with ceric sulfate solution.

Plant Material

The leaves of *F. nitida* were procured from Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi.

Extraction and isolation

The leaves (1 kg) were coarsely powdered and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (102.4 g, 10.2%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over a silica gel column (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively with various combinations of petroleum ether, chloroform and methanol in order of increasing polarity. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the leaves of *F. nitida*:

Stigmasterol (1)

Elution of the column with petroleum ether – chloroform (3 : 1) gave colourless amorphous solid of **1**, 117 mg; m.p. 162 164 °C, UV λ_{max} (MeOH): 211 nm (log ε 5.8); IR υ_{max} (KBr): 3425, 2920, 2852, 1641, 1463, 1373, 1225, 1173, 801 cm $^{-1}$; ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 5.16 (1H, m, H-22), 5.01 (1H, m, H-23), 3.65 (1H, brm, $w_{1/2}$= 16.5 Hz, H-3α), 2.23 to 1.23 (25 H, m, 9 x CH_2. 7 x CH), 1.05 (3H, brs, Me-19), 0.96 (3H, d, J=6.3 Hz, Me-21), 0.87 (3H, d, J=6.6 Hz, Me-26), 0.84 (3H, d, J=6.0 Hz, Me-27), 0.80 (3H, t, J=6.6 Hz, Me-29), 0.71 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 36.52 (C-1), 31.90 (C-2), 71.81 (C-3), 41.90 (C-4), 140.76 (C-5), 121.69 (C-6), 31.66 (C-7), 33.94 (C-8), 51.24 (C-9), 37.26 (C-10), 21.07 (C-11), 39.76 (C-12), 42.30 (C-13), 56.87 (C-14), 24.17 (C-15), 28.67 (C-16), 55.96 (C-17), 12.24 (C-18), 19.41 (C-19), 36.68 (C-20), 18.79 (C-21), 138.30 (C-22), 129.28 (C-23), 45.83(C-24), 27.28 (C-25), 19.83 (C-26), 18.99 (C-27), 23.11 (C-28), 12.05 (C-29), EIS MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (30.2), 394 (100).

β-Sitosterol (2)

Elution of the column with chloroform yielded colourless amorphous powder of **2**, 98 mg; R_f 0.35 (chloroform: methanol: 9: 1); m. p. 137-138 ^{o}C; UV λ_{max} (MeOH): 211 nm (log ε 2.9); IR υ_{max} (KBr): 3401, 2918, 2849, 1654, 1377, 1261, 1172, 1082 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (1H, m, H- 6), 3.54 (1H, brs, $w_{½}$= 18.5 Hz, H- 3), 1.01 (3H, brs, M- 19), 0.94 (3H, d, J= 6.2 Hz, Me- 21), 0.87 (3H, d, J= 6.5 Hz, Me- 27), 0.84 (3H, J= 6.3 Hz, Me- 26), 0.82 (3H, t, J=6.1 Hz, Me- 29), 0.67 (3H, brs, Me-18), 2.28 – 1.05 (29H, 11 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ

37.28 (C- 1), 31.93 (C- 2), 71.81 (C- 3), 42.34 (C- 4), 140.78 (C- 5), 121.68 (C- 6), 29.33 (C- 7), 34.23 (C- 8), 50.21 (C- 9), 36.14 (C- 10), 22.66 (C- 11), 38.89 (C- 12), 39.81 (C- 13), 56.80 (C- 14), 27.21 (C- 15), 28.22 (C- 16), 56.11 (C- 17), 11.85 (C- 18), 19.33 (C- 19), 36.73 (C- 20), 19.03 (C- 21), 33.98 (C- 22), 26.18 (C- 23), 45.90 (C- 24), 29.68 (C- 25), 21.07 (C- 26), 19.78 (C- 27), 24.94 (C- 28), 11.97 (C- 29); +ve ion FABMS *m/z* (rel.int.): 414 $[M]^+$ ($C_{29}H_{50}O$) (15.1), 398 (100), 383 (13.5).

Friedelin (3)

Further elution of the column with chloroform afforded colourless amorphous solid of **3**, 71 mg; m.p. 258–260 °C; IR γmax: 2937, 2848, 1709, 1639,1453, 1379, 1263, 1183, 1077, 1012, 987, 924 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.27 (3H, s, Me-28), 1.18 (3H, s, Me-27), 1.03 (3H, s, Me-26), 0.93 (3H, s, Me-29), 0.89 (3H, d, J = 6.3 Hz, Me-23), 0.86 (s, 3H, H-30), 0.80 (s, 3H, H-25), 0.71 (s, 3H, H- 24), 2.42 – 0.91 (26 H, m, 4 x CH, 22 x CH_2); ^{13}C NMR (CDCl3): δ 22.33 (C-1), 41.58 (C-2), 213.27 (C-3), 58.19 (C-4), 42.23 (C-5), 41.28 (C-6), 18.36 (C-7), 53.08 (C-8), 37.44 (C-9), 59.56 (C-10), 35.6 3(C-11), 30.49 (C-12), 38.27 (C-13), 39.74 (C-14), 32.39 (C-15), 36.13 (C-16), 30.18 (C-17), 42.77 (C-18), 35.38 (C-19), 28.23 (C-20), 32.76 (C-21), 39.23 (C-22), 6.78 (C-23), 14.74 (C-24), 18.07 (C-25), 20.25 (C-26), 18.67 (C-27), 32.15 (C-28), 35.09 (C-29), 31.84 (C-30); ESI MS *m/z* (rel. int.): 426 ($[M]^+$ ($C_{30}H_{50}O$) (6.6), 411 (2.3), 218 (14.8), 205 (14.6), 191 (15.2).

β-Amyrin (4)

Elution of the column with chloroform – methanol (49:1) eluent furnished crystals of **4**, recrystallized from chloroform– methanol (1:1), 64 mg; m. p. 198° C, R_f = 0.63 (benzene–chloroform, 4:1) (lit. m.p. 197–198° C); Leibermann–Burchard test positive; IR γmax (KBr): 3358, 2925, 2843, 1635, 1455, 1035, 981 cm^1; ^{1}H NMR ($CDCl_3$): δ 5.27 (1H, m, H-12), 3.14 (1H, dd, J = 5.6, 9.3 Hz, H-3β), 1.14 (3H, s, Me-23), 1.08 (3H, s, Me-25), 1.04 (3H, s, Me-28), 0.98 (3H, s, Me-24), 0.95 (3H, s, Me-26), 0.88 (6H, s, Me-29), 0.83 (3H, s, Me-30), 0.78 (3H, s, Me-27), 2.01- 1.17 (23 H, 10 x CH_2 , 3 x CH); ^{13}C NMR ($CDCl_3$): δ 38.32 (C-1), 22.27 (C-2), 80.64 (C-3), 37.29 (C-4), 55.34 (C-5), 18.71 (C-6), 32.66 (C-7), 39.90 (C-8), 47.63 (C-9), 36.96 (C-10), 23.53 (C-11), 121.53 (C-12), 145.38 (C-13), 41.83 (C-14), 26.29 (C-15), 26.23 (C-16), 32.53 (C-17), 47.37 (C-18), 46.89 (C-19), 31.2 2 (C-20), 34.86 (C-21), 37.13 (C-22), 16.78 (C-23), 28.47 (C-24), 15.51 (C-25), 16.25 (C-26), 25.92 (C-27), 23.40 (C-28), 33.39 (C-29), 19.47 (C-30); EIS MS *m/z* 426 $[M]^+$ ($C_{30}H_{50}O$) (12.6).

Ficusesquaterpene (5)

Elution of the column with chloroform-methanol (19:1) mixture produced a colourless powder of **5**, recrystallized from acetone, 1.8 g. m.p.: 274 – 275 °C, UV λmax (MeOH): 207 nm (log ε 3.8), IR γmax (KBr): 2928, 2847, 1456, 1365, 1258, 1041, 928 cm^{-1}, ^{1}H NMR (CDCl3) δ 2.35 (1H, dd, J = 4.9, 7.5 Hz. H-9), 2.30 (1H, dd, J = 4.8, 8.6 Hz, H-17), 2.26 (1H, dd, J = 5.6, 9.2 Hz, H-21), 1.73 (1H, J = 5.3, 8.8 Hz, H-13), 1.65 (1H, dd, J = 5.8, 10.3 Hz, H-5), 1.98 – 1.29 (28H, m, 14 x CH2), 1.30 (3H, brs, Me-27), 1.27 (3H, brs, Me-35), 1.22 (6H, brs, Me-29, Me-34), 1.12 (3H, brs, Me-33), 1.04 (3H, brs, Me-32), 1.09 (3H, brs, Me-31), 0.98 (3H, brs, Me-28), 0.76 (3H, brs, Me-30); ^{13}C NMR ($CDCl_3$): δ 39.25 (C-1), 29.69 (C-2), 32.43 (C-3), 38.38 (C-4), 53.21 (C-5), 18.31 (C-6), 33.36 (C-7), 40.07 (C-8), 42.84 (C-9), 37.48 (C-10), 22.27 (C-11), 35.33 (C-12), 29.33 (C-13), 42.13 (C-14), 29.63 (C-15), 29.48 (C-16), 34.98 (C-17), 59.51 (C-18), 35.64 (C-19), 36.06 (C-20), 30.05 (C-21), 36.06 (C-22), 32.51 (C-23), 32.06 (C-24), 32.55 (C-25), 41.32 (C-26), 20.23 (C-27), 16.77 (C-28), 14.64 (C-29), 14.05 (C-30), 17.92 (C-31), 28.17 (C-32), 22.65 (C-33), 18.62 (C-34), 28.16 (C-35); ESI MS (+ve mode) *m/z* (rel. int.): 480 $[M]^+$ (C35H60) (7.2), 465 (11.3), 450 (14.7), 220 (32.6), 192 (37.8), 152 (34.3), 124 (26.4).

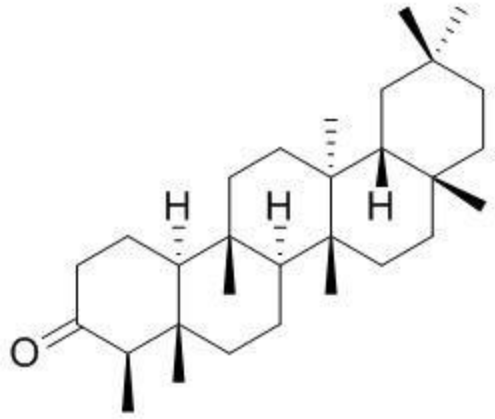
RESULTS AND DISCUSSION

Compounds **1** – **4** were the known phytoconstituents characterized as stigmasterol, β-sitosterol, friedelin and β-amyrin, respectively[10. 17 - 19].

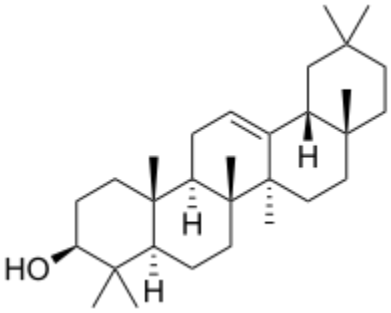
Compound **5,** named ficusesquaterpene, was obtained as a colourless powder from chloroform – methanol (19:1) eluants. Its IR spectrum was devoid of any functional group and vinylic bonds.

On the basis of it mass and ^{13}C NMR spectra, its molecular ion peak was established at *m/z* 480 consistent with a molecular formula of a sesquaterpene $C_{35}H_{60}$. The important ion peaks arising at *m/z* 124 [$C_{5,6}$ – $C_{9,10}$/$C_{17,22}$ – $C_{20,21}$ fission]$^+$ and 152 [$C_{7,8}$ – $C_{9,10}$ fission]$^+$ indicated saturated nature of the rings A, B and F. The ion peaks appearing at *m/z* 192 [$C_{8,14}$ – $C_{9,11}$/$C_{16,17}$ – $C_{13,18}$ fission]$^+$ and 220 [$C_{8,14}$ – $C_{12,13}$ fission]$^+$ supported devoid of any vinylic linkage in the rings C and D. The ^{1}H NMR spectrum of

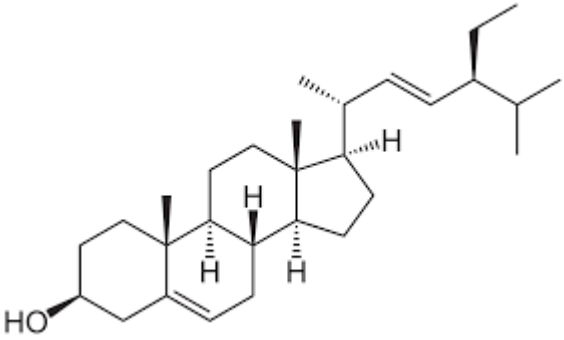
5 displayed seven three-proton singlets at δ 1.30, 1.27. 1.12, 1.04, 1.09, 0.98 and 0.76 and a six-proton singlet at δ 1.22 assigned correspondingly to tertiary C-27, C-25, C-33, C-32, C-31, C-28, and C-30 and C-34 and C-29 methyl protons, all of them were attached to the saturated carbons. Five one-proton double doublets at δ 2.35 (J = 4.9, 7.7 Hz), 2.30 (J = 4.8, 8.8 Hz), 2.26 (J = 5.6, 9.2 Hz), 1.73 (J = 5.3, 8.8 Hz) and 1.66 (J = 5.8, 12.3 Hz) were ascribed to methine H-9, H-17, H-21, H-13 and H-5 protons, respectively. The ^{13}C NMR spectrum of **5** displayed signals for 35 carbon signals. The methine carbon signals resonated at δ 53.12 (C-5), 40.84 (C-9), 29.30 (C-13), 34.98 (C-17) and 30.05 (C-21); the methyl carbons appeared between δ 28.17 – 14.05. The DEPT spectrum of **5** showed the presence of nine methyl, 14 methylene, five methine and seven quaternary carbons. On the basis of the foregoing account the structure of **5** has been established as sesquaterpene. It contains a hexacyclic ring structure.



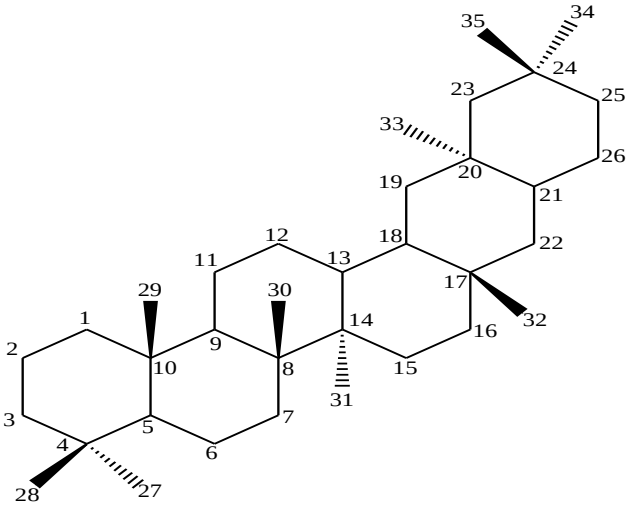
Friedelin (3)



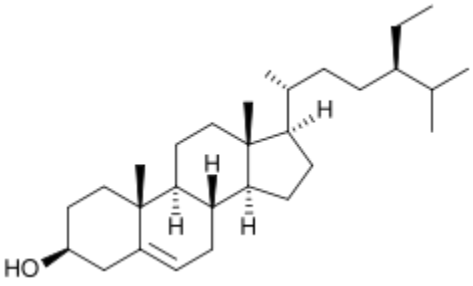
β-Amyrin (4)



Stigmasterol (1)



Ficusesquaterpene (5)



β- Sitosterol (2)

CONCLUSION

Phytochemical investigation of a methanolic extract of the leaves of *F. nitida* resulted in the isolation of a new sesquaterpene (ficusesquaterpene) along with the known compounds stigmasterol, β-sitosterol, friedelin and β-amyrin . This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the leaves.

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REFERENCES

1. U. Quattrocchi, CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology , CRC Press Inc, Boca Raton, Florida, 2012; 1733.
2. Flora of Pakistan, Flora of Pakistan/Pakistan Plant Database (PPD). Tropicos website St. Louis, Missouri and Cambridge, Massachusetts, USA: Missouri Botanical Garden and Harvard University Herbaria, 2014.
3. NCSU, Poisonous Plants website, USA: North Carolina State University, 2014 .
4. S. Hasti , M. Mora, R. Utami, L.U.Yulis, Sub-chronic Toxicity of *Ficus benjamina* L. Leaves Ethanol Extract on the Liver Function of White Mice . Procedia Chemistry, 13, 2014, 204-208.
5. S.P. Parajuli, Ethnobotanical studies of at Khandbari muncipilty of Sankhuwasabha. Banko Janak. 2000, 10, 29-34.
6. V.K. Kanaujia, H.K. Rirchhaiya, S.D. Kailasiya, M. Verma, R.D. Yadav, D. Shivhare, Evaluation of hepatoprotective activity on the leaves of *Ficus benjamina* Linn. J Nat Prod Plant, 2011, 1, 59-69.
7. N. Sirisha, M. Sreenivasulu, K. Sangeeta, C.M. Chetty, Antioxidant properties of *Ficus* species, a review. Int J Pharma Techn Res, 2010, 4, 2174-2182.
8. H.A. Almahyl, M. Rabmanj, M.A. Sukari, A.M. Ali, The chemical constituents of Ficus benjamina Linn. and their biological activities. Pertanika J Sci Techno, 11(1), 73 - 81 (2003).
9. M. Imran, N. Rasool, K. Rizwan, M. Zubair , M. Riaz , M. Zia-Ul-Haq, U.A. Rana, A. Nafady , H..Z.E. Jaafar, Chemical composition and Biological studies of *Ficus benjamina.* Chemistry Central Journal 2014, **8,** 12; **DOI:** 10.1186/1752-153X-8-12.
10. M. Parveen, R.M Ghalib, S.H. Mehdi, S.Z. Rehman, M. Ali. A new triterpenoid from the leaves of Ficus benjamina (var. comosa). Nat. Prod. Res., 2009, 23, 729-736.
11. M. Parveen, R.M. Ghalib, S.H. Mehdi, M. Ali , A novel antimicrobial triterpenic acid from the leaves of Ficus benjamina (var. comosa), J. Saudi Chem. Soc, 2009, 13, 287- 290 .
12. I.A. Ogunwande, R. Jimoh, A.A. Ajetunmobi, N.O. Avoseh, G. Flamini, Essential oil composition of *Ficus benjamina* (Moraceae) and I*rvingia barteri* (Irvingiaceae). Nat Prod Commun, 2012, 7, 1673-1675.
13. J. Dai, D. Shen, W.Y. Yoshida, S.M. Parrish, P.G. Williams, Isoflavonoids from Ficus benjamina and their inhibitory activity on BACE1. Planta Med, 2012, 78, 1357-1362.
14. L. Yarmolinsky, M. Huleihel, M. Zaccai, S. Ben-Shabat, Potent antiviral flavone glycosides from *Ficus benjamina* leaves. Fitoterapia. 2012, 83, 362-367. 10.1016/j.fitote.2011.11.014.
15. S. Novelli, C. Lorena, C. Antonella, Identification of Alkaloid's Profile in Ficus benjamina L. Extracts with Higher Antioxidant Power. American Journal of Plant Sciences, 2014, 5, 4029- 4039.
16. R.C. Da Cruz, V. Agertt., A.A. Boligon, V. Janovik, M.M. Anraku de Campos, D. Guillaume , M.L. Athayde, In vitro antimycobacterial activity and HPLC-DAD screening of phenolics from Ficus benjamina L. and Ficus luschnathiana (Miq.) Miq. leaves. Nat Prod Res, 2012, 26 (23), 2251-4.
17. G.F. Sousa, L.P. Duarte , A.F.C. Alcântara, G.D.F. Silva, S.A. Vieira-Filho, R.R. Silva , D.M. Oliveira, J.A. Takahashi , New Triterpenes from Maytenus robusta: structural elucidation

based on NMR experimental data and theoretical calculations. Molecules 2012, 17, 13439-13456. doi:10.3390/molecules171113439

18. J.S.S. Quintans, E.V.C. Costa , J.F. Tavares , T.T. Souza, S.S. raújo , C.S. Estevam , Barison , A.G.S. Cabral , M.S. Silva , M.R. Serafini, L.J. Quintans-Júnio , Phytochemical study and antinociceptive effect of the hexanic extract of leaves from *Combretum duarteanum* and friedelin, a triterpene isolated from the hexanic extract, in orofacial nociceptive protocols. Rev Bras Farmacogn, 2014, 24, 60-66.
19. C.E. Ogwuche, J.O. Amupitan, G. Ndukwel, R.G. Ayo, Isolation and biological activity of the triterpene β-amyrin from the aerial plant parts of Maesobotrya Barteri (Baill). Med Chem, 2014, 4 (11), 729-733.