APIGENIN-4', 7-DIMETHYL ETHER FROM *AQUILARIA AGALLOCHA* ROXB.

ABSTRACT

Earlier researchers have reported the presence of several sesquiterpenoids, chromones and alkaloids. In the present investigation isolation and characterization of a flavone namely apigenin-4', 7-dimethyl ether for the first time from the wood of *Aquilaria agallocha* Roxb.

Keywords: *Aquilaria agallocha*, Flavones, Apigenin-4', 7-dimethyl ether.

INTRODUCTION

Agaru wood/Eagle Wood/(Tamil:Akil)is botanically equated as *Aquilaria agallocha* Roxb. (syn. *A. malaccensis* Lam.) Fam. Thymeliaceae. A large evergreen 20 to 30 m high fungus infected tree producing maximum amount of dark coloured resinous fragrant heart wood from 50 to 60 years old tree growing in hilly forest of Assam, Meghalaya, Nagaland, Manipur and Tripura. The wood occurs in hard, moderately heavy, longitudinally split pieces of varying size, 4 to 7 cm long and 3 to 5 cm thick. The surface is faintly, longitudinally striated white lines representing medullary rays, fracture is hard, colour creamish yellow, odour and taste are nil. Ethyl acetate extract of heartwood showed *in vitro* antioxidant activity with oxidation of haemoglobin in human blood haemolysate[1]. Studies on the antibacterial activity of the methanol and aqueous extracts of leaf and bark showed that aqueous extract of bark and leaf produced inhibition zones in *Shigella flexneri* and *Pseudomonas aeruginosa*. Methanol extract of leaf showed inhibitory effect against *Bacillus subtilis*[2]. Earlier workers have reported the following constituents; (5*S*, 6*S*, 7*R*, 8*S*)-2-(2-phenylethyl-6, 7, 8-trihydroxy-5, 6, 7, 8-tetrahydro-5-[2-(2-phenylethyl) chromonyl-7-hydroxyl -6-oxy] chromone, bi-(5*S*, 6*S*, 7*R*, 8*S*)-2-(2-phenylethyl-6, 7, 8-trihydroxy-5, 6, 7, 8-tetrahydro-5-[2-(2-phenylethyl) chromonyl- 6, 7-dioxy] chromone[3], liriodenine[4], agarol, gmelofuran[5], aquillochin[6], 2-(2-4'-methoxyphenylethyl) chromone[7], 6-methoxy-2-(2-4'-methoxyphenylethyl) chromone[8], agarotetrol[9, 10], isoagarotetrol[10], 6-hydroxy-2-(2-phenylethyl) chromone, 6-methoxy-2-(2-phenylethyl) chromone), 6-methoxy-2-[2-(3-methoxyphenyl) ethyl] chromone, 6, 7-dimethoxy-2-(2-phenylethyl) chromone[11], (5*S*, 6*S*, 7*R*, 8*S*)-2-(2-phenylethyl-6,7,8-trihydroxy-5,6,7,8-tetrahydro-5-[2-(2-phenylethyl) chromonyl-6-oxy]chromone, 2,2'-di-(2-phenylethyl)-8, 6'-dihydroxy-5, 5'-bichromone[12], (-)-guaia-1(10), 11-dien-15-al, (-)-selina-3, 11-dien-9-one, (+)-selina-3,11-dien-9-ol[13], 5 α,6 β,7 β,8 α-tetracetoxy-2-[2-(4-methoxy-phenyl)ethyl]-5,6,7,8- tetrahydrochromone, 5α,6β,7α,8β-tetrahydroxy-2-[2-(2-hydroxy-phenyl) ethyl]-5, 6, 7, 8-tetrahydrochromone, 5 α, 6 β, 7 α, 8 β-tetrhaydroxy-2-[2-(4-methoxy-phenyl)ethyl]-5, 6, 7, 8-tetrahydrochromone[14], jinkohol [15], (-)selina-3, 11-dien-14-al, (+)selina-4,11-dien-14-al, (-)-methyl selina-3,11-dien-14-oate, (+)selina-4,11-dien-14-oate,(+) methyl 9-hydroxy selina-4, 11-dien-14-oate, (+)- 1, 5-epoxy-nor-ketoguaiene, dihydrojinkoh-cremol and neopetasane[16], α–agarofuran, (-)-10-epi-γ eudesmol and oxoagarospiral[17], (-) guaia-1(10),11-dien-15-ol, (-) guaia-1(10), 11-dien-15-carboxylic acid, methylguaia-1(10), 11-dien-15- carboxylate, (+) guaia-1(10),11-dien-9-one, (-)-1.10-epoxyguai-11-ene, (-) guaia-1(10),11-dien-15, 2-olide and (-)-rotundone[18], α-agarofuran, β-agarofuran, nor-keto agarofuran, (-)-10-epi- γ eudesmol, agrospiral, jinkohol, jinkoh-cremol, kusunol, dihydrokaranone, jinkohol II, oxo- agrospiral[19]. In the present study characterization of apigenin-4', 7-dimethyl ether (Fig. 1) is reported for the first time from the wood of *A. agallocha*.

EXPERIMENTAL

Wood of *A. agallocha* was collected from Regional Research Institute for Ayurveda, Guwahati, unit of Central Council for Research in Ayurvedic Sciences, New Delhi and authenticated by Dr. Jeyaraman, Plant

Anatomy Research Centre, Tambaram, Chennai. A voucher specimen of the sample no: 241 was deposited in the Pharmacognosy department of the institute. Coarsely powdered wood (1.5 kg) was extracted with *n*-hexane by cold percolation method for 72 h. The extraction was repeated and filtered. The combined extract was concentrated by distilling over a boiling water bath. The last traces of the solvent were removed under vacuum. Extract (7.4821g) was column chromatographed using silica gel (acme 100-200 mesh; 1:22) as the stationary phase and eluted with solvents of increasing polarity such as *n*-hexane, chloroform, ethyl acetate and methanol and their mixtures. Earlier fractions yielded waxy material. Fractions eluted with chloroform, on removal of the solvent gave a solid which on crystallization yielded a compound. The TLC examination of the compound showed a single spot at R_f 0.59 in the mobile phase of toluene:ethyl acetate (9:1). The compound had a sharp melting point at 165°, answered positively for phenol and flavonoid.

IR ν_{max} (KBr) cm^{-1}: 3448 (hydroxyl) 3050, 2988, 2848, 1640 (chelated carbonyl), 1604, 1508 (aromatic), 1445, 1382, 1339, 1246, 1162, 1018, 833.

UV λ_{max}^{MeOH} 270, 328 nm

^{1}H NMR, δ_{ppm} $CDCl_3$ (400 MHz): 6.36 (1 H, s, J= 2.2Hz, H-8); 6.48 (1H, d, J=2.2 Hz, H-6); 6.57(1H, s, H-3); 7.02 (2H, d J=8.8 Hz, H-3' & H-5'); 7.85 (2H, d, J=8.8 Hz, H-2'and H-6'); 3.88 (3H, s, 4'-OCH_3); 3.89 (3H, s, 7-OCH_3); 12.81 (1H, brs,5-OH)

^{13}C NMR δ_{ppm} $CDCl_3$ (100.62 MHz): 92.62 (C-8), 98.04 (C-6), 104.35 (C-3), 105.56 (C-10),114.50 (C-3' & C-5'), 123.57(C-1'), 128.04 (C-2' & C-6'); 162.19 (C-9), 157.7 (C-5), 162.60 (C-2), 164.02 (C-4'), 165.43 (C-7), 182.45 (C-4), 55.53 (4'-OCH_3), 55.79 (7-OCH_3).

RESULTS AND DISCUSSION

The compound m.f. $C_{17}H_{14}O_5$, m.p. 165° gave positive ferric reaction for phenol and Shinado's test for flavones. IR spectrum showed the presence of hydroxyl (3448) and chelated flavone carbonyl (1640) cm^{-1}. In the UV spectrum there were maxima at 270 and 328 nm. Addition of NaOAc did not produce any appreciable shift in band II showing the absence of 7-OH. The absence of ortho dihydroxy function was shown as there was no bathochromic shift on adding boric acid. Addition of $AlCl_3$ showed a shift of 50 nm in band I which was not altered on adding conc. HCl. Thus showing the presence of 5-hydroxyl and absence of 3-hydroxyl function. Addition of NaOMe did not produce any appreciable shift in band I revealing the absence of 4'-hydroxyl.

In the ^{1}H NMR spectrum of the compound, two methoxy groups (C-4' and C-7) appeared as three proton singlets at δ 3.88 and 3.89 respectively. The B ring showed A2B2 pattern with two proton doublets at δ 7.02 (H-3'and H-5') and 7.85 (H-2' and H-6') with J=8.8 Hz. H-3 appeared as one proton singlet at δ 6.57. The two meta coupled protons H-6 and H-8 appeared as doublets (J=2.2 Hz) at δ 6.48 and 6.36. The chelated hydroxyl at C-5 appeared downfield as a one proton broad singlet at δ 12.81. These data suggested the structure of the compound to be 4', 7-dimethoxy 5-hydroxy flavone (apigenin-4', 7-dimethyl ether [1]). The ^{13}C NMR data also confirmed the structure of the compound (see experimental).The identity of the compound was confirmed by comparison of the physical and spectroscopic data with those reported in literature[20].

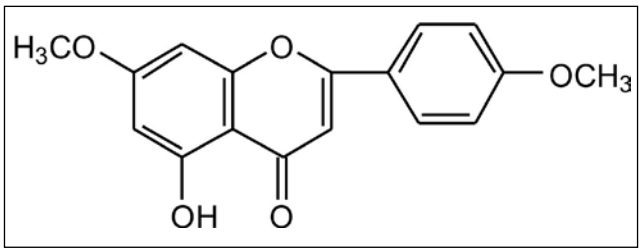

Fig. 1: Apigenin-4', 7-dimethyl ether [1]

ACKNOWLEDGEMENT

Director General, Central Council for Research in Ayurvedic Sciences, Dept. of AYUSH, Ministry of

Health & Family Welfare, Govt. of India, New Delhi for financial support.

REFERENCES

1. The Ayurvedic Pharmacopoeia of India, Part-1, vol. IV. 1st edn. Government of India, Ministry of Health and Family Welfare, Department of AYUSH, New Delhi: 2004, 4-5.
2. Dash M., Patra J. K., Panda P. P.: Phytochemical and antimicrobial screening of extracts of *Aquilaria agallocha* Roxb., **Afr J Biotech.** 2008, 7(20), 3531-3534.
3. Iwagoe K., Kodama S., Konishi T., Kiyosawa S., Fujiwara Y. and Shimada Y.: The structures of AH_{15} and AH_{16}, new bi- and tri- phenylethylchromones from agalwood, **Chem Pharm Bull.** 1987, 35(11), 4680- 4682.
4. Natrajan A. K., Natarajan M., Purushothaman K. K.: Alkaloids from Agaru, **Bull Med Ethno Bot Res.** 1983, 4(1&2), 81-84.
5. Pant P., Rastogi R. P.: Agarol, a new sesquiterpene from *Aquilaria agallocha*, **Phytochemistry.** 1980, 19(8), 1869-1870.
6. Bhandari P., Pant P., Rastogi R. P.: Aquillochin, a coumarinolignan from *Aquilaria agallocha*, **Phytochemistry.** 1982, 21(8), 2147-2149.
7. Hashimoto K., Nakahara S., Inoue T., Sumida Y., Takahashi M., Masada Y.: A new chromone from agarwood and pyrolysis products of chromone derivatives, **Chem Pharm Bull.** 1985, 33(11), 5088-5091.
8. Nakanishi T., Inada A., Nishi M., Yamagata E., Yoneda K.: A new and a known derivative of 2-(2-phenylethyl) chromone from a kind of agarwood ("Kanankoh", in Japanese) originating from *Aquilaria agallocha*, **J Nat Prod.** 1986, 49(6), 1106-1108.
9. Yoshii E., Koizumi T., Orbie T., Takeuchi F., Kubo K.: The structure of agarotetrol, a novel highly oxygenated chromone from agarwood (Jinko), **Tetrahedron Lett.** 1978, 19(41), 3921-3924.
10. Shimada Y., Konishi T., Kiyosawa S., Nishi M., Miyahara K., Kawasaki T.: Studies on the agalwood (Jinko). IV[1]). Structures of 2-(2-phenylethyl) chromone derivatives, agarotetrol and isoagarotetrol, **Chem Pharm Bull.** 1986, 34(7), 2766-2773.
11. Shimada Y., Tominaga T., Konishi T., Kiyosawa S.: Studies on the agarwood (Jinko). I. Structures of 2-(2-phenylethyl) chromone derivatives, **Chem Pharm Bull.** 1982, 30(10), 3791-3795.
12. Iwagoe K., Konishi T., Kiyosawa S., Shimada Y., Miyahara K., Kawasaki T.: The structures of AH_{10} and AH_{11}, nobel bi-phenylethylchromones from agalwood, **Chem Pharm Bull.** 1986, 34(11), 4889-4891.
13. Ishiharaa M., Tsuneya T., Shigab M., Uneyamab K.: Three sesquiterpenes from agarwood, **Phytochemistry.** 1991, 30(2):563-566.
14. Shimada Y., Konishi T., Kiyosawa S.: Studies on the Agalwood (Jinko). VI[1]. Structures of three 2-(2-phenylethyl)-5, 6, 7, 8- tetrahydrochromone derivatives, $AH_{1A,}$ AH_{2a} and AH_{2b}, **Chem Pharm Bull.** 1986, 34(7), 3033-3037.
15. Nakanishi T., Yamagata E., Yoneda K. and Miura I.: Jinkohol, a prezizane sesquiterpene alcohol from agarwood, **Phytochemistry.** 1981, 20(7), 1597-1599.
16. Ishiharaa M., Tsuneya T. and Uneyama K.: Fragrant sesquiterpenes from agarwood, **Phytochemistry.** 1993, 33(5), 1147-1155.
17. Nakanishi T., Yamagata E., Yoneda K., Nakashima T., Kawasaki I., Yoshida T., Mori H. and Miura I.: Three Fragrant sesquiterpenes from agarwood, **Phytochemistry.** 1984, 23(9), 2066-2067.
18. Ishiharaa M., Tsuneya T. and Uneyama K.: Guaiane sesquiterpenes from agarwood, **Phytochemistry.** 1991, 30(10), 3343-3347.
19. Yoneda K., Yamagata E., Nakanishi T., Nakashima T., Kawasaki I., Yoshida T., Mori H. and Miura I.: Sesquiterpenoids in different kinds of agarwood, **Phytochemistry.** 1984, 23(9), 2068-2069.
20. Ahmad Reza Gohari, Hakimeh Ebrahimi, Soodabeh Saeidnia, Mahdi Foruzani, Panueh Ebrahimi and Yousef Ajani: Flavones and flavones glycosides from *Salvia macrosiphon* Boiss, **Iran J Pharm Res.** 2011, 10(2), 247-251.

Saraswathy A.*, Vidhya B. and Amala K.

Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS)
Anna Hospital Campus, Arumbakkam,
Chennai-600106, Tamil Nadu.
E-mail: csmdria@gmail.com; saraswathy20002004@gmail.com

*For correspondence

(Received 02 May 2012) (Accepted 06 August 2012)