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Phytochemical And Antimicrobial Investigations On MUSSAENDA GLABRATA

KEYWORDS: Mussaenda frondosa, Rubiaceae, rutin, Escherichia coli, and Bacillus subtili

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ABSTRACT *Mussaenda is a genus of flowering plants in the Rubiaceae family. They are native to the African and Asian tropics and subtropics. Several species are cultivated as ornamental plants. It contains some 194 species. Mussaenda frondosa syn. Mussaenda glabrata popularly known in Siddha/Tamil Vellai-yilai, Velli- madandai. The fresh flowers have been found to contain the flavonol glycoside : rutin. The structure of the isolated yellow pigment has been characterized by means of modern physical methods like UV, 1H-NMR, 13C-NMR, chemical reactions, hydrolytic studies and chromatographic investigations. The isolated rutin is found to contain considerable anti-inflammatory activity.*

INTRODUCTION

Mussaenda frondosa syn. Mussaenda glabrata of Rubiaceae found in tropical Himalayas, Khasi Hills, Deccan Peninsula and the Andamans. Different parts of this plant are used as diuretic, antiasthmatic, antiperiodic. Leaves and flowers are used in external applications for ulcers. Root is used in the treatment of white leprosy. The flowers contain anthocyanins, hyperin, quercetin, ferulic, sinapic acid and beta-sitosterol glucoside. An infusion of the leaves is used for cough, asthma, recurrent fevers and also as a diuretic in dropsy[1]. The roots of Mussaenda glabrata are found to contain radical scavenging and antistress activity[2]. Phytochemicals like astragalin, isoquercetin, kaempferol-3-o-beta drutinoside were isolated from leaves[3]. A new compound sanzhilactone along with mussaenside,barlerine lupeol and beta-d-glucose has been obtained from the stem[4].

EXPERIMENTAL

Extraction and fractionation:

Fresh flowers (1 kg) of M. glabrata collected from in and around Kumbakonam during June were extracted with 85% EtOH (4 X 500 ml) under reflux. The alc. extract was concentrated in-vacuo and the aq. concentrate successively fractionated with benzene (3 X 250 ml), peroxide-free Et_2O (3 X 250 ml) and EtOAc (4 X 250 ml).

No crystalline solid could be recovered from benzene and Et_2O fractions.

EtOAc fraction : (flavonol glycoside : rutin)

The residue from EtOAc fraction was taken up in Me_2CO and left in an ice-chest for two days when a pale yellow solid separated. It came out as pale yellow plates on recrystallization, m.p. 187-89 °C, yield 0.1% and developed a greenish-brown colour with alc.Fe^{3+}, formed yellow precipitate with basic lead acetate solution and reduced ammoniacal $AgNO_3$ but not Fehling's solution. It had the Rf values of the yellow pigment rutin. It responded to Wilson's boric acid[5], Molisch's and Gibbs'[6] tests but did not answer the Horhammer – Hansel-[7]test. It had λnm 257, 281, 297 sh, 360; (+NaOMe) 273, 325, 411; (+$AlCl_3$) 273, 305 sh, 435; (+$AlCl_3$/HCl) 269, 301, 364, 399; (+NaOAc) 273, 301 sh, 313, 377; (+NaOAc/H_3BO_3) 265, 301, 372. It was identified as rutin and the identity confirmed by co- and mixed-PC and m.m.p with an authentic sample of Wrightia tinctoria[8].

Hydrolysis of the glycoside:

The glycoside (0.05 g, 0.2 m mole) dissolved in hot aq. MeOH (2 ml, 50%) was hydrolysed with H_2SO_4 (5%) at 100 °C for about 2 hr and the hydrolytic products identified as described below.

Identification of the aglycone :(flavonol: quercetin)

The aglycone on recrystallisation from methanol gave yellow leaflets, m.p. 316 - 18 °C (yield 0.02%) which was identified as quercetin by colour reactions, behaviour under UV and R_f (Table I-5). The Et_2O fraction was concentrated in vacuo and left in an ice chest for about a week. A yellow solid that separated was filtered and studied. It came out as pale yellow needles m.p. 316-18 °C on recrystallization from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive-green colour with NH_3 and NaOH, yellow solution with a pale green fluorescence with conc. H_2SO_4 and appeared yellow under UV and

UV/NH_3. It answered Wilson's boric acid, Horhammer – Hansel and Gibbs' tests but did not respond to Molisch's test. It had λnm 255, 269 sh, 301 sh, 370; (+NaOMe) 247 sh, 321 (dec.); (+$AlCl_3$) 272, 304 sh, 333, 458; (+$AlCl_3$/HCl) 265, 301 sh, 359, 428; (+NaOAc) 257 sh, 274, 329, 390; (+NaOAc/H_3BO_3) 262, 304 sh, 388 and had R_f values of quercetin. The ^1H- and ^{13}C-NMR of the flavonol are appended. It was identified as quercetin and the same was confirmed by co- and mixed-PC and m.m.p with and authentic sample of quercetin from Physalis minima[9].

Identification of the sugar: (glucose and rhamnose)

The filtrate after the removal of the aglycone was neutralized with $BaCO_3$. The concentrated filtrate when examined by paper chromatography gave R_f values corresponding to those of glucose and rhamnose. The running properties of the glycoside were also in favour of a bioside. The identity of the sugars was confirmed by comparison with authentic samples of glucose and rhamnose.

Partial hydrolysis of the glycoside:

The glycoside was subjected to partial hydrolysis by treatment with 10% formic acid in cyclohexane[10, 11]. The resulting solution was extracted with ethyl acetate and subjected to PC. The R_f values of the EtOAc fraction agreed with those of quercetin-3-O-rutinoside (rutin). On this basis it can be concluded that glucose is directly linked to the aglycone moiety.

Antimicrobial activity of the glycoside

During this investigation the antimicrobial activity of the isolated quercetin 3-O-rutinoside, has been analysed, using E. coli a Gram negative microorganism and B. subtilis a Gram positive organism. The nephloturbidity meter has been employed for the determination of the antimicrobial activity. Streptomycin has been used as a reference.

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RESULTS AND DISCUSSION

The flowers of M. glabrata have been found to contain rutin (quercetin-3-O-rutinoside).

The UV spectrum of the glycoside showed two absorption maxima at 360 nm (band I) and 257 nm (band II). A bathochromic shift of 51 nm observed in band I of its NaOMe spectrum indicates the presence of a free –OH group at C-4′. The $AlCl_3$-HCl spectrum of the glycoside showed four absorption maxima indicating a free –OH group at C-5 which is further supported by a bathochromic shift of 39 nm in its $AlCl_3$-HCl spectrum and positive response to Wilson's boric acid test. The presence of a –OH group at C-7 could be inferred from a bathochromic shift of 16 nm (band II) on the addition of NaOAc. The presence of a catechol type of B-ring could be inferred from a bathochromic shift of 12 nm (in band I) noticed in its NaOAc- H_3BO_3 spectrum. Further, a bathochromic shift observed in the MeOH spectrum (band I) of the aglycone obtained after hydrolysis of the glycoside as compared to that of the glycoside suggests that the site of glycosylation could be at C-3 which is also supported by the fact that the glycoside did not respond to the Horhammer – Hansel test whereas the aglycone did.

In the ^{1}H-NMR spectrum of the glycoside (400 MHz, DMSO-d_6, TMS) the signal at δ 7.97 ppm (d, J = 9 Hz) and 7.56 ppm (J = 6 Hz) correspond to the protons at C-2′ and C-6′ respectively. The proton at C-5′ appears at δ 6.84 ppm (d, J = 8 Hz) whereas those of C-6 and C-8 resonate respectively at δ 6.19 ppm (d, J = 1.7 Hz) and 6.39 ppm (d, J = 2.0 Hz). the 5 – OH proton appears at δ 12.61 ppm. H-1″ of glucose resonates at δ 5.4 ppm (J =8 Hz) while that of H-1‴ of rhamnose at δ 4.57 ppm (d, J = 4 Hz)[12]. The signal appearing in the range of δ 0.8 – 1.1 ppm correspond to the C-6‴ protons (methyl protons of rhamnose) and is clearly reminiscent of the presence of rutinoside[13]. Had it been a neohesperidoside where the linkage is 1→2, the corresponding signal would have appeared at δ 1.1 – 1.3 ppm. The rest of the sugar protons appear in the range of δ 3.0 - 3.8 ppm[14].

Supporting evidence for the structure of the flavonol glycoside is provided by the ^{13}C-NMR (100 MHz, DMSO-d_6, TMS) spectral data. Due to glycosylation, the signal of C-3 is shifted upfield by 2.60 ppm. The downfield shift of the ortho related C-2 signal by 9.40 ppm also confirms this[15]. The large shift in C-2 resonance also reflects the semi-olefinic character of the flavonol C-2, C-3 double bond[16]. The signal at δ 104.0 ppm of C-10 is less intense due to the longer relaxation time of the quaternary carbon[17]. The signal of C-6‴ of rhamnose at δ 17.5 ppm (not at δ 20.90) and that of C-6″ signal at δ 67.9 ppm (not at δ 60.90) clearly shows that the glycoside is a 3-O-rutinoside[18].

On this basis, the glycoside from the EtOAc fraction can be characterized as quercetin-3-O-rutinoside (rutin). The isolated yellow pigment is more active against E. subtilis than E. coli. The selective inhibition of growth of Gram-positive bacteria has been observed among most of the antibiotics.[19]

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REFERENCE

1. C.P. Khare, Indian medicinal plants- An illustrated dictionary, Springer, 2007, 295, 471. | 2. Sameksha Koul and Anu Chaudhary, Pharmacologyonline, 2011, 1, 1091. | 3. Ranarivelo, Y.A.L Skaltsounis, M.Andriantsiferana and F.Tillequin, Glycosides from Mussaenda arcuata Lam.ex Poiret leaves.Ann Pharm Fr., 1990, 48(5), 273. | 4. Biswanath Dinda, Sudhan Debnath and Santanu Majumder, Chemical constituents of Mussaenda incana, Ind J Chem., 2005, 44B (11), 2362. | 5. C. W. Wilson, J. Amer. Chem. Soc., 1928, 48. | 6. J. Shinoda, J. Chem. Pharm. Soc., 1928, 48. | 7. L. Horhammer and R. Hansel, Arch. Pharm. Berl., 1955, 288, 315. | 8. V. Sethuraman, M. G. Sethuraman, N. Sulochana and R. A. Nambi, Indian Drugs, 1984, 22, 158. | 9. V. Sethuraman and N. Sulochana, Fitoterapia, 1988, 59, 335. | 10. T. A. Geissman, The Chemistry of Flavonoid Compounds, Pergamon Press, London, 1962, 99. | 11. D. W. Fox, W. L. Sakvage and S. H. Wender, J. Amer. Chem . Soc., 1953, 75, 2504. | 12. H. Rosler, T. J. Mabry, M. F. Cranner and J. Kagan, J. Org. Chem.,1965, 30, 4346. | 13. K. R. Markham and T. J. Mabry, in 'The Flavonoids', J. B. Harborne, T. J. Mabry and H. Mabry (Eds.), Chapman and Hall, London, 1975, 70. | 14. H. Rosler, T. J. Mabry, M. F. Cranner and J. Kagan, J. Org. Chem.,1965, 30, 4346. | 15. B. Ternai and K. R. Markham, Tetrahedr., 1976, 32, 565. | 16. G. C. Levy and G. L. Nelson, carbon-13 Nuclear Magnetic Resonance for Chemists,, Wiley, New York, 1972, 63. | 17. K. R. Markham and V. M. Chari, The flavonoids – Advances in Research, Chapman and Hall, London, 1982, 22. | 18. B. G. Oesterdha, Acta. Chem. Scand., 1978, B32, 714), (K. R. Markham, B. Ternai, R. Stanley, H. Geiger and T. J. Mabry, Tetrahedr., 1978, 34, 1389. | 19. J.S.Glassby, 'Encyclopedia of antibiotics', John wiley, London, 1978, 372. |