An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

CHEMICAL EXAMINATION OF THE LEAVES OF *MILLETTIA PULCHRA (BENTH.) KURZ*

Kiran S Divi[1], Krishna. N[1] and Ganapaty .S*[2]

[1]Divis Laboratories Ltd, Chippada, Visakhapatnam-531162 (A.P.)
[2]GITAM Institute of Pharmacy, GITAM (Deemed University), Rushikonda, Visakhapatnam- 530 045 (A.P.)

***Corresponding author E-mail: **ganapatyseru@gmail.com**

ARTICLE INFO

Key Words
Millettia pulchra Kurz.(Fabaceae), Triterpenes, srerols and flavonoids

ABSTRACT

Chemical analysis of *Millettia pulchra* leaves (Fabaceae) afforded Friedelin, β-sitisterol, Stigmasterol, auriculasin, scandenone and 6,8-diprnylorobol. The compounds were identified by chemical tests, chromatographic analysis and spectroscopy.

INTRODUCTION:

Millettia pulchra (syn: *M. taiwania*)[1] is perennial climbing shrub and is a Fabaceae member. It is one of the most well known among 150 species of *Millettia,* and is widely used in traditional practices, such as agriculture pesticide, blood tonic and treatment of cancer and infertility. It is also recorded to possess estrogenic, insecticidal, anthelmintic and fish-poisoning properties. Earlier, Erysenegalensein E, Euchrenone F, Isoerysenegalensein E, 6,8-diprenylorobol, Furowanin A and B, Millewanin F, G and H, Scandenone, and Auriculasin were reported from the leaves of *M. pulchra*[2,3].

Material and Methods: The leaf material *M. pulchra* (1.5 kg) were collected from tribal pockets of North coastal districts of Andhra Pradesh, India. The leaf material was, air dried at room temperature 37˚C and extracted with chloroform (3 x 1.5 L). After concentrating the combined extract under reduced pressure, 15 g green residue was obtained. The extract gave dark green colour with ferric chloride indicating the presence of phenolic compounds and also positive pink colour with Liebermann-Burchard test indicating presence of triterpenes. TLC analysis of *Millettia pulchra* extract showed five prominent spots in chloroform:hexane (1:19) on spraying with 5% ethanolic sulphuric acid. A part of the extract (10 g) was chromatographed with gradient elution successively with n-hexane, chloroform and methanol. During elution, six compounds were separated and were designated as **MPLC-1** to **MPLC-6.**

Characterization of the compounds MPLC-1: (0.1 g, Friedelin): The compound was crystallized from chloroform: hexane (1:19) as white needles, m.p. 263-265°C, $[\alpha]_D^{20}$ (*c*, 0.595 in $CHCl_3$) -25.1°. It was analyzed for the formula $C_{30}H_{50}O$. It gave play of colours (pink to blue to green) to Liebermann-Burchard test. A 2,4- dinitro phenylhydrazone derivative of the compound showed m.p. 301-303°C and was analyzed for the formula $C_{36}H_{54}N_4O_4$. The properties of the compound **MPLC-1** and its derivative closely resemble to those of friedelin and its corresponding 2,4-dinitrophenylhydrazone derivative. Hence, the compound was identified as friedelin.

MPLC-2: (0.08 g, β-sitosterol)

It was crystallized from petroleum ether as colourless needles, m.p. 137-139°C, $[\alpha]_D^{30}$ (*c*, 1.01 in $CHCl_3$) -36.0°. It was analyzed for the formula $C_{29}H_{50}O$. It responded to Liebermann-Burchard test for sterols with a play of colours (pink to blue to green). IR spectrum showed peaks at 3436 (O-H stretch), 1384 and 1378 cm^{-1}. The ^1H NMR displayed signals at δ 0.68-1.25 (6 x Me); 3.47 (1H, m, H-3); 5.32 (1H, m, H-6); From the above data, the compound **MPLC-2** was identified as β-sitosterol. Further identity of the compound was confirmed by comparison with an authentic sample through m.m.p. and co-TLC.

MPLC-3: (0.1 g, Stigmasterol)

It was crystallized from hexane and obtained as white feathery needles, m.p. 163-165°C, $[\alpha]_D^{30}$ (*c*, 1.123 in $CHCl_3$) -37.0°. It was analyzed for the formula $C_{29}H_{48}O$. It gave positive pink colour with Liebermann-Burchard and dense red ring with Salkowski test for sterols. IR showed absorption bands at 3412 (O-H stretch), 1636 (C=C stretch), 1170, 1111 (C-O stretch), 1058, 990, 971 (vinyl group), and 936 cm^{-1}. From the above characteristics, it was identified as stigmasterol and was confirmed by comparing with an authentic sample through m.m.p and co-TLC.

MPLC-4: (0.04 g, Auriculasin): It was crystallized from chloroform as pale yellow needles, m.p. 174-176°C, It was analyzed for the formula $C_{25}H_{24}O_6$. It gave dense green colour with ferric chloride. The **UV** spectrum showed n: 225 and 298. The **^1H NMR** (400 MHz, $CDCl_3$) displayed signals at δ 7.88 (1H, *s*, H-2); 13.98 (1H, *s*, H-5-OH); 6.94 (1H, *d*, *J*=1.5 Hz, H-2′); 6.42 (2H, *br s*, H-3′- and H-4′-OH); 6.78 (1H, *d*, *J*=8.1 Hz, H-5′); 6.70 (1H, *dd*, *J*=8.1, 1.5 Hz, H-6′); 1.46 (6H, *s*, H-2″ Me); 5.60 (1H, *d*, *J*=10 Hz, H-3″); 6.74 (1H, *d*, *J*=10 Hz, H-4″); 3.38 (2H, *d*, *J*=7.2 Hz, H-1‴); 5.16 (1H, *t*, *J*=7.0 Hz, H-2‴); 1.80 (3H, *s*, H-4‴, Me-cis); 1.69 (3H, *s*, H-5‴, Me-trans). From the above characteristics, the compound was identified as auriculasin. It was confirmed by comparison with the authentic sample through m.m.p and co-TLC.

MPLC-5: (0.05 g, Scandenon: It was crystallized from chloroform: petroleum ether (1:19) mixture as shining yellow needles, m.p. 164-165°C. It was analyzed for the formula $C_{25}H_{24}O_5$. It gave dark green colour with ferric chloride. The UV spectrum showed nm: 225 and 287. The ^1H NMR (400 MHz, $CDCl_3$,) showed a signals at δ 7.88 (1H, s, H-2); 13.00 (1H, s, H-5-OH); 7.30 (2H, d, J=8.4 Hz, H-2′ and H-6′); 6.78 (2H, d, J=8.4 Hz, H-3′ and H-5′); 6.03. (1H, s, H-4′-OH); 5.62 (1H, d, J=10 Hz, H-3″); 6.73 (1H, d,J=10 Hz, H-4″); 1.46 (6H, s, H-2″-Me); 3.39 (2H, d, J=7.2 Hz, H-1‴); 5.17 (1H, t, J=7 Hz, H-2‴); 1.81 (3H, s, H-4‴, Me-cis); 1.68 (3H, s, H-5‴, Me-trans). From the above spectral properties, MPLC-5 was identified as scandenone. Further identity was confirmed by comparison with an authentic sample through m.m.p. and co-TLC.

MPLC-6: (0.45 g, 6, 8-diprenylorobol): It was crystallized from chloroform as pale yellow needles, m.p. 154-155°C and was analyzed for the formula $C_{25}H_{26}O_6$. The UV spectrum showed nm: 274. The ^1H NMR (400 MHz, DMSO-d_6) showed signals at δ 7.88 (1H, s, H-2); 12.91 (1H, s, H-5OH);

6.92 (1H, s, H-7-OH); 6.94 (1H, d, J=1.5 Hz, OH); 6.82 (1H, *d, J*=8.1 Hz, H-5′); 6.75 (1H, *dd*, *J*=8.1, 1.5 Hz, H-6′); 3.42 (2H, *m*, H-1″); 5.25 (1H, *m*, H-2″); 1.83 (3H, *s*, H-4″, Me-cis); 1.73 (3H, *s*, H-5″, Me-trans); 3.45 (2H, *m*, H-1‴); 5.22 (1H, *m*, H-2‴); 1.80 (3H, *s*, H-4‴, Me-cis); 1.69 (3H, *s*, H-5‴, Me-trans). From the above characteristics, the compound was identified as 6,8- diprenylorobol. Further identity was confirmed by comparison with the authentic sample through m.m.p and co-TLC.

Experimental

Plant material:

The leaf material *Millettia pulchra* was collected from the tribal pockets of North coastal districts of Andhra Pradesh State. It was authenticated by Dr. M.. Venkaiah, Taxonomist, Dept of Botany, Andhra University, Visakhapatnam. A voucher specimen (KSG003) was deposited at Herbarium, of the University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

Extraction:

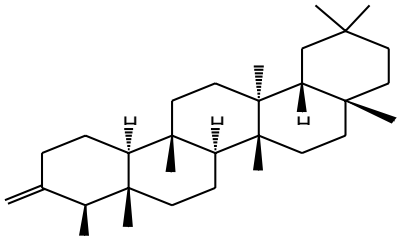
1 kg of dried leaf powder of *Millettia pulchra* was extracted with chloroform (3 x 1.5 L). TLC examination of the residue showed five prominent spots in chloroform: hexane (1:19). The combined extract was concentrated under reduced pressure and 16 g thick green residue was yielded. 10 g of the extract was chromatographed on silica gel and successively eluted (each 200 ml fraction) with n- hexane, chloroform and methanol.

Elution and isolation:

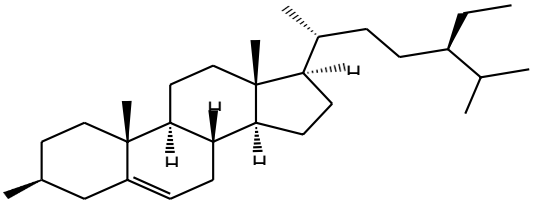
Elution of the chromatogram with chloroform: hexane (25:75) (fractions 33-38) and repeated crystallization with hexane and chloroform, it afforded white needles MPLC-1 (0.1 g) and was identified as friedelin. Further elution with a solvent system, chloroform: hexane (25:75) (fractions 39-44) yielded a colourless amorphous powder, which on further purification with petroleum ether afforded H-2′); 6.41 (2H, br s, H-3′- and H-**4′-** colourless needles, MPLC-2 (0.08 g) and was identified as β-sitosterol. Further elution with chloroform and hexane (fractions 45-48) yielded white needles and on crystallization from hexane feathery needles of MPLC-3 (0.1 g) was obtained and was identified as stigmasterol. Elution with chloroform: hexane (40:60) (fractions 53-58) yielded yellow needles. Repeated crystallization from chloroform afforded pale yellow needles of MPLC-4 (0.04 g and was identified as auriculasin. Elution with chloroform: hexane (50:50) (fractions 62-64) yielded yellow needles and was further purified by crystallization with petroleum ether and chloroform, an yellow shining needles of MPLC-5 (0.05 g) was obtained and was identified as scandenone. On continuation of elution with chloroform: hexane (55:45) (fractions 65-69) yielded yellow needles. Repeated crystallization with chloroform another compound MPLC-6 (0.45 g) was obtained and was identified as 6, 8-diprenylorobol.

RESULTS AND DISCUSSION

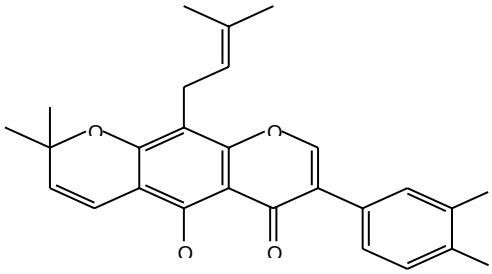
Isoflavonoids have very limited distribution in the plant kingdom, and the plants belong to Fabaceae are the major source of these compounds. The potential applications of isoflavonoids from M. pulchra in developing new pharmaceutical agents based on folkloric anecdotes and evidences from pharmacological and biochemical assays encourages further research into their pharmacological applications[4-6]. Due to estrogenic, insecticidal, anthelmintic and fish-poisoning properties of different morphological parts of Millettia pulchra, they were investigated to find out number of bioactive compounds. Chemical analysis of M. pulchra leaves on conventional gradient chromatographic separation afforded six compounds namely friedelin (MPLC-1), β-sitosterol (MPLC-2), stigmasterol (MPLC-3), auriculasin (MPLC-4), scandenone (MPLC-5) and 6,8-diprenylorobol (MPLC-6).



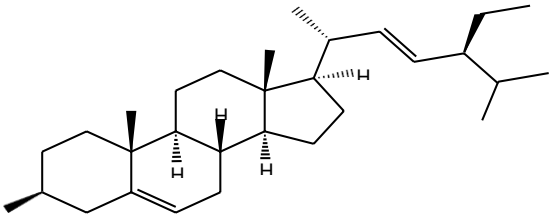
(**MPLC-1**) Friedelin



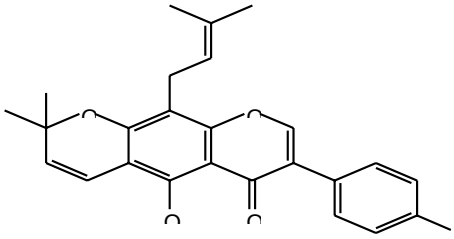
(***MPLC-2) β-sitosterol***



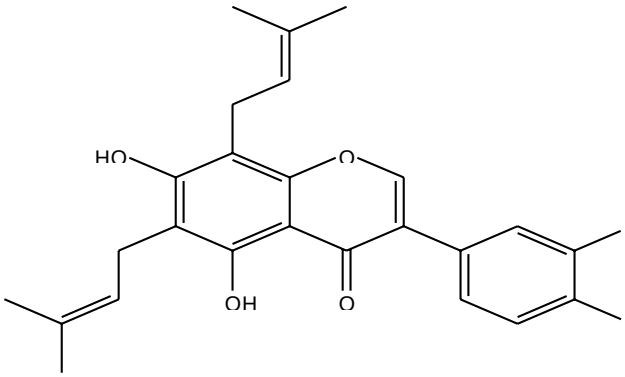
(**MPLC-3**) Stigmasterol



(MPLC-4) Auriculasin



(**MPLC-5**) Scandenone



(**MPLC-6**) 6,8-diprenylorobol

All the compounds were identified by chemical tests and spectral data. A number of bio-active chemicals have been reported from *Millettia pulchra* including several rotenoids, prenylflavonoids, dihydroflavonol and chalcones from the seed. Rotenoids such as tephrosin, deguelin, 6a, 12a-dehydrodeguelin and 13-homo-13-oxa-6a,12a-dehydrdeguelin; pyranoisoflavones like 4′,5′-dimethoxy-6,6-dimethyl-1-pyranoisoflavone and barbigerone were isolated from seed. Rotenone, cis-12a-hydoxyrotenone, rot-2′-enoic acid and cis-12a-hydroxyrot- 2′-enoic acid were isolated from the root. Several chemical analyses yielded a number of prenylated isoflavones including Erysenegalensein E, Euchrenone b10, Isoerysenegalensein E, 6,8-diprenylorobrol, Furowanin A and B, Millewanin F, G and H, Scandenone and Auriculasin from the leaf. The major flavonoid component of the stem was found to be auriculasin[7-9].

Acknowledgements:

The authors are grateful to GITAM Management for providing necessary facilities to carry out the above work. They are also thankful to University Scientific Instrumentation Centre (USIC), Andhra University for providing NMR Spectra of some of the compounds.

REFERENCES:

1. Chopra R, Nayar S, Chopra I Glossary of Indian Medicinal Plants, New Delhi, CSIR; 1956, 105.
2. Ito C, Itoigawa M, Kojima N, Tokuda H, Hirata T, *et al*. Chemical constituents of *Millettia taiwaniana*: structure elucidation of five new isoflavonoids and their cancer chemopreventive activity. *J. Nat. Prod*. 2004;67(7):1125-30.
3. Ito C, Itoigawa M, Kumagaya M, Okamoto Y, Ueda K, *et al*. Isoflavonoids with antiestrogenic activity from *Millettia pulchra. J Nat Prod*. 2006;69(1):138- 41.
4. Ito C, Murata T, Itoigawa M, Nakao K, Kumagai M, *et al*. Induction of apoptosis by isoflavonoids from the leaves of *Millettia taiwaniana* in human leukemia HL-60 cells. *Planta Med*. 2006;72(5):424-9.
5. Yoshinori O, Atshushi S, Koji U, Ito C, Ito M. *et al.* Anti-estrogenic activity of prenylated isoflavones from *Millettia pachycrapa*: implications from pharmacophores and unique mechanisms. *Journal of Health Sciences.* 2006;52(2):186-91.
6. Ye H, Chen L, Li Y, Peng A, Fu A, Song H, *et al*. Preparative isolation and purification of three rotenoids and one isoflavone from the seeds of *Millettia pulchra (Benth.) Kurz* by

high-speed counter-current chromatography. *J Chromatogr A*. 2008;1178(1-2):101-7.

7. Singhal AK, Sharma RP, Baruah JN, Govindan SV, Herz W. Rotenoids from roots of *Millettia pulchra.Phytochemistry.*1982;21(4): 949-951.

8. Singhal AK, Sharma RP, Thyagarajan G, Herz W, Govindan SV. New prenylated isoflavones and a prenylated dihydroflavonol from *Millettia pulchra. Phytochemistry.*1980;19(5):929-934.

9. Ye H, Zhong S, Li Y, Peng A, Hu J, Shi J, *et at.* Enrichment and isolation of barbigerone from *Millettia pulchra (Benth.) Kurz*. Using high-speed counter-current chromatography and preparative HPLC. *Journal of Separation science.* 2010; 33(8):1010-1017.