Research Article

ANTIMYCOBACTERIAL CHROMONE RHAMNOSIDE FROM *MIMOSA PIGRA* LINN. (FABACEAE) AERIAL PART

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ABSTRACT

Activity guided fractionation of the aerial part methanol extract of *Mimosa pigra* resulted in the isolation and characterization of a bioactive chromone rhamnoside (MIC; 0.5 mg/mL) as an anti-mycobacterial constituent. The structure of the bioactive chromone rhamnoside was elucidated using spectroscopic techniques to be 8-hydroxy-3-(phenyl)-4-benzopyrone-6-O- rhamnoside. This is the first report of anti-mycobacterial activity of *Mimosa pigra* and its potentials for the development of drug leads for the treatment of tuberculosis thus validating its uses in ethno-medicine.

Key Words: Chromone rhamnoside, *Mimosa pigra*, tuberculosis, drug discovery, Ethno-medicine

INTRODUCTION

From time immemorial, plants and plant-derived drugs have been used as traditional remedies for the treatment of diseases¹⁻². Over two-third of the world population still depend on herbal medicine for its healthcare needs³. This number is still growing daily due to the high cost of orthodox therapy especially in the third world countries. Among the infectious diseases, Tuberculosis (TB) is one of the common and often deadly. It is caused by *Mycobacterium tuberculosis*. The prevalence of multi-drug resistant tuberculosis is an increasing health challenge. The World Health Organization (WHO) reported 8.6 million new cases of TB in 2012 with associated deaths due to MDR-TB put at 170, 000 worldwide⁴. Less developed countries, mostly in Africa and Asia accounted for the highest burden. Attention is therefore been shifted to the use of ethno-medicines in combating this disease. The use of plants in ethno medicine for the treatment of tuberculosis and related respiratory tract infections has been documented⁵. *Mimosa pigra* is a legume of the genus *Mimosa* and family Fabaceae. It is a shrub reaching up to 6 m in height. It is called “giant sensitive plant,” “Kai dafi” in Hausa, and “Enwa agogo” in Yoruba. The stem which is armed with broad-based prickles up to 7 mm long, is greenish in young plants but becomes woody as the plant matures⁶. The Leaves are sensitive and fold up when touched and at nightfall. It can be distinguished from its closely related species *Mimosa pudica* (commonly called sensitive plant) by its large size, large pods (6 to 8 cm long as opposed to 2.5 cm long), and leaves which have 6 to 16 pairs of pinnae as opposed to 1 to 2 pairs in *Mimosa pudica* leaves⁶. Its use in the traditional treatment of asthma and other respiratory diseases has been reported⁷. Among the Bawku in Upper East Ghana, it is used in the treatment of diarrhea, typhoid fever and genitourinary tract infections⁸. Its anti-hypertensive⁹ and anti-oxidant¹⁰⁻¹¹ activities have also been reported. The flavonoids; Kaempferol, quercetin, myricetin and their glycosides have been isolated from the leaves of *Mimosa pigra*^{10,12}.

MATERIAL AND METHODS

General experimental procedures

Solvents used were analytical grade product of Sigma Aldrich and purchased from Zayo Chemicals Nigeria Ltd, the Sigma Aldrich accredited agent in Nigeria. Column chromatography was performed on silica gel (Merck 70-230) under gravity. Thin layer chromatography (TLC) was performed on plates pre-coated with silica gel 60 HF₂₅₄ (Merck, TLC grade, with gypsum binder). The TLC bands were visualized by exposure to iodine and by spraying with concentrated H₂SO₄ using spray gun. Solvents were removed under reduced pressure using a Buchi rotary evaporator at pump pressure of 0.1 mmHg. The ¹H and ¹³C NMR spectra were recorded at 200 MHz (50 MHz for ¹³C NMR analysis) on a Bruker model spectrometer in deuterated CDCl₃. Chemical shifts are expressed in parts per million (ppm) downfield of Trimethylsilane (TMS) as internal reference for ¹H resonances, and referenced to the central peak of the appropriate deuterated solvent's resonances. Infra red (IR) spectra were recorded on 1600 ATI Matson Genesis series FTIRTM spectrometer. Mass spectra were recorded on a FINNIGAN MAT 12 spectrometer. The melting points were recorded on an electrothermal melting point apparatus and are uncorrected. The TB Research Laboratory facilities at The Zankli Medical Centre, Abuja, Nigeria were used for the antimycobacterial assay. De-contaminated clinical strain of *Mycobacterium tuberculosis* obtained from the same laboratory was used for the investigation.

Sample collection, identification and processing

Mimosa pigra aerial parts used for this study were collected from farmlands in Chaza village, Suleja, Niger State, Nigeria in the month of November 2010, by Mallam Muazam and authenticated at the herbarium unit of the National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria. Voucher specimens; NIPRD/H/6405 have been deposited in the herbarium of

the Institute. The plant materials were sorted out to remove humus, washed and dried under ambient condition. The dried plant materials were then pulverized into fine powder and kept in air tight polythene bags prior to extraction.

Extraction procedure

The dried powdered aerial part of *M. pigra* (500 g) was extracted by cold maceration with intermittent agitation for 72 hours in absolute methanol (3 x 2.5 L). The vacuum dried methanol extract was suspended in methanol: water (1:9 v/v) and partitioned with diethyl ether and n-butanol in succession.

Antimycobacterial assay procedures

The solid based agar dilution (using the egg enriched Lowenstein Jensen (LJ) medium), and the liquid based Mycobacterium Growth Indicator Tube (MGIT) methods were used at the test concentration range of 5 – 0.5 mg/mL. Colony counting was done as reported by the International Union against Tuberculosis LJ Medium (IUTM)¹³⁻¹⁴ as outlined below:

- No growth at the end of incubation period at 37 °C
- + 1-19 colonies growth units at the end of incubation period at 37 °C
- 1+ 20-100 colonies growth units at the end of incubation period at 37 °C
- 2+ 100-200 colonies growth units at the end of incubation period at 37 °C
- 3+ 200-400 colonies growth units at the end of incubation period at 37 °C

Where a test substance showed inhibition with greater than '+' colonies, it is taken as not being active. Where a no colony growth was observed, the test substance is taken as bactericidal.

Phytochemical Methods

Phytochemical tests for alkaloids, triterpenoids, steroids, tannins, flavonoids, free reducing sugars, saponins and glycosides were carried out on the solvent fractions and isolated constituents following standard methods¹⁵⁻¹⁶.

Column chromatography of the n-butanol fraction of *M. pigra* (aerial part)

The n-butanol fraction (0.9 g) from the crude methanol extract of *M. pigra* aerial part was dissolved in methanol and pre-adsorbed on silica gel (0.9 g) by mixing to form a homogenous paste which was allowed to air dry in a fume cupboard. The mixture was separated using column chromatography (Length = 30 cm, internal diameter = 2 cm and weight of silica gel (mesh 60 -230) = 100 g). The column was eluted with the following gradient of mobile phase: ethyl acetate: methanol (10:0, 150 mL), ethyl acetate: methanol (9:1, 100 mL), and ethyl acetate: methanol (8:2, 100 mL). A total of 15 fractions (20 mL portions using pre-calibrated glass sample bottles) were collected and pooled to based on TLC analysis. The pooled fractions eluted with ethyl acetate: methanol (10:0 (4th – 5th eluate), to 9:1(6th – 10th eluate)) afforded a light yellow solid coded MPL after washing with diethyl ether and re-crystallisation in acetone.

Characterization of MPL

Appearance: light yellow solid

Melting point: 148 -152°C

Phytochemistry: positive to FeCl₃ and Molisch tests

Molecular mass (calculated): 400.3787 g/mol.

EI-Mass spectrum: (m/z): 399[M-H], 318(25) [M-ring B], 302 (55) [M-15-ring B], 286(64) 258(5), m/z 184 [due to ring C cleavage], and base peak at 153(100).

¹³C-NMR (50 MHz, DMSO-d₆ δ ppm): Rhamnose moiety : 102.6 (d, C-1'), 70.7 (d, C-2'), 71.1 (d, C-3'), 71.3 (d, C-4'), 72.0 (d, C-5') and 19.0 (q, C-6'), chromone ring : 94.7 (d, C-2), 137.2 (s, C-3), 178.5 (s, C=O, C-4), 99.4 (d, C-5), 164.9 (s, C-6), 108.6 (d, C-7), 161.9 (s, C-8), 120.3 (s, C-9), and 157.1 (s, C-10), and aryl ring B: 145.6 (s, C-1'') and 116.0 (d, C-2', C-6' of monosubstituted aromatic ring B). The results are presented in Table 1.

¹H-NMR (200 MHz, DMSO-d₆, δ ppm): Rhamnose moiety : 5.2 (1H, d, H-1' due to anomeric proton), 3.1-3.9 (4 x 1H, m, H-2', H-3', H-4', H-5') and 0.9 (3H, d, J=5.8 Hz, H-6'), chromone ring: 7.3 (1H, H-2), and two meta coupled aromatic protons 6.2 (1H, H-5) and 6.4(1H, H-7), aryl ring B : 6.9 ppm (5H, mono-substituted aromatic ring B, H-2', H-6'). The results are presented in Table 1

FTIR (V_{max} cm⁻¹, Nujol): 3280 (OH), 2920, 2850 (CH), 1657 (C=O), 1576, (Aromatic C=C), 969, 827.31, 717(Aromatic C=C deformation) and 1167 (C-O).

UV/Visible spectrum (λ_{max} nm, methanol): 217, 233, 335.

Compound MPL (8-hydroxy-3-(phenyl)-4-benzopyrone-6-O- rhamnoside)

Table 1: Rationalized ^1H and ^{13}C NMR (CDCl_3) spectra data of compound MPL

S/No	δ_c ppm	APT	δ_H ppm
1	-	-	-
2	94.7	CH	7.3
3	137.2	C	-
4	178.5	C=O	-
5	99.4	CH	6.2
6	164.9	C	-
7	108.6	CH	6.4
8	161.9	-	-
9	120.3	-	-
10	157.1	-	-
1'	145.6	-	-
2'	116.0	CH	6.9
3'	116.0	CH	6.9
4'	116.0	CH	6.9
5'	116.0	CH	6.9
6'	116.0	CH	6.9
1''	102.6	CH	5.2 (1H, d, J= 5.2 MHz)
2''	71.1	CH	3.1-3.9
3''	71.3	CH	3.1-3.9
4''	72.0	CH	3.1-3.9
5''	70.7	CH	3.1-3.9
6''	19.0	CH ₃	0.9 (3H, d, J= 5.8 MHz)

om = overlapping multiplet, s = singlet proton or C, d = doublet proton or CH, t = triplet proton or CH₂, q = quartet proton or CH₃

Scheme 1: EI-MS fragmentation scheme for MPL from *M. pigra* aerial part

RESULTS AND DISCUSSIONS

Whereas the n-butanol fraction showed bactericidal effect at the test concentration of 2.0 mg/mL against the clinical strain of *M. tuberculosis*, its parent crude methanol extract exhibited bactericidal activity only at the highest test concentration of 5.0 mg/mL. Column chromatography separation of the bioactive n-butanol fraction resulted into the isolation of the compound MPL (MIC 0.5 mg/mL). Compound MPL (melting point: 148 - 152°C) gave a single spot (R_f = 0.65) on TLC examination with CHCl_3 : MeOH (3:1) as mobile phase. The MPL gave a positive test observation with 10% alcoholic FeCl_3 reagents. This is an indication that it is a phenolic compound. The EI-MS showed the base peak at m/z 153. This is typical of flavonoids with hydroxylation at ring A¹⁷. It is due to cleavages at ring C, in addition to loss of the sugar residue. The ^{13}C -NMR (50 MHz, $\text{DMSO}-d_6$, δ_c ppm) indicated a total of seventeen carbon signals which on the basis of the Attached Proton Test (APT) analysis reflected for; one methyl, nine methine (one of which is due

to the five equivalent methine of the mono-substituted aryl ring B), six quaternary and one carbonyl. The trend in δ_c (ppm) signals: 102.62 (d, OCHO, C-1''), 70.70 (d, CHOH, C-2''), 71.1 (d, CHOH, C-3''), 71.3 (d, CHOH, C-4''), 72.0 (d, CHOH, C-5'') and 19.0 (q, CH₃, C-6'') is a characteristic of a rhamnose residue¹⁸⁻¹⁹. Rhamnose is a methyl hexose sugar. This supported MPL to be a rhamnoside. In addition the δ_c (ppm) signals: 94.7 (d, C-2), 137.2 (s, C-3), 178.5 (s, C=O, C-4), 99.4 (d, C-5), 164.9 (s, C-6), 108.6 (d, C-7), 161.9 (s, C-8), 120.3 (s, C-9), and 157.1 (s, C-10), 145.6 (s, C-1') and 116.0 (d, C-2', C-6' of monosubstituted aromatic ring B,) are evident for an aryl benzopyrone (or aryl chromone) nucleus typical of the C-6-C-3 biosynthetic origin of flavonoids and related phenolics secondary metabolites like the coumarines and phenylpropanoids. The quaternary carbon signal at 178.5 ppm corresponding to C-4 is indicative of the C=O functionality of the chromone. The ^1H -NMR (200 MHz, $\text{DMSO}-d_6$) spectrum showed the overlapping proton multiplet peaks in the region 3.1-3.9 ppm typical of sugar residue and the corresponding anomeric proton doublet signal at δ_H : 5.21

ppm (1Hd, J =5.2 MHz), and one upfield methyl doublet proton 0.9 ppm (3H, d J=5.8 MHz) due to CH₃ of rhamnose unit). This corroborated similar observation in the ¹³C-NMR spectrum. Rhamnose is a methyl deoxy monosaccharide which has resonance for the C₆ methyl protons as a doublet upfield in addition to four ring secondary carbinol and one anomeric proton signal²⁰. The δ_H (ppm) resonance for the two meta coupled aromatic protons 6.18 (H₅) and 6.38 (H₇) in addition to that at 6.9 (5H, H₂-H₆) due to the mono-substituted phenyl ring B of the flavonoid nucleus were also seen. After comparing the spectra data with that simulated using ACD software, the chemical shift assignment for compound MPL (8-hydroxy-3-(phenyl)-4-benzopyrone-6-O- rhamnoside) was done as shown in Table 1. The FTIR_{nujol} spectrum: 3280.34 cm⁻¹(OH), 2919.71 cm⁻¹, 2850.29 cm⁻¹ (CH), 1656.56 cm⁻¹ (C=O), 1575.56 cm⁻¹ (Aromatic C=C), 968.49 cm⁻¹, 827.31 cm⁻¹, 717.39 cm⁻¹ (Aromatic fingerprinting), and 1166.72 cm⁻¹ (C-O) is indicative of a phenol with cyclic carbonyl moiety. UV-Visible λ_{max}: 217, 233, 335 nm are indication of the conjugated chromane or benzopyran system typical of flavonoids and related naturally occurring compounds²¹. Flavonoids are of immense pharmacological importance. The anti-diarrhea activity of quercetin, an ubiquitous flavonoid, has been reported²². The insecticidal, fish poisoning, anaesthetic, and anti-inflammatory activities of rotenone -an isoflavonoids, have been reported²³. The antiviral²⁴, anti-oxidant properties²⁵⁻²⁷ and antibacterial²⁸ activities of flavonoids have also been reported.

FURTHER STUDIES

Further work is on-going to confirm the proposed structure of the compound MPL using two-dimensional NMR spectroscopic techniques and X-Ray crystallography, and also to investigate non-antimycobacterial fractions for other pharmacological activities based on other ethno-medicinal uses of the plant.

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