Fitoterapia 93 (2014) 189–193

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

# Synthesis and antibacterial evaluation of 3-Farnesyl-2-hydroxybenzoic acid from *Piper multiplinervium*

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ARTICLE INFO

*Article history:*
Received 15 April 2013
Accepted in revised form 31 December 2013
Available online 18 January 2014

*Keywords:*
3-Farnesyl-2-hydroxybenzoic acid
Prenylated salicylic acid
*Piper multiplinervium*
*Staphylococcus aureus*
Antibacterial

ABSTRACT

3-Farnesyl-2-hydroxybenzoic acid is an antibacterial agent isolated from the leaves of *Piper multiplinervium*. This compound has activity against both Gram positive and Gram negative bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Helicobacter pylori*. This research aimed to synthesize a natural antibacterial compound and its analogs. The synthesis of 3-Farnesyl-2-hydroxybenzoic acid consists of three steps: straightforward synthesis involving protection of phenolic hydroxyl group, coupling of suitable isoprenyl chain to the protected aromatic ring at *ortho* position followed by carboxylation with concomitant deprotection to give the derivatives of the salicylic acid. All the three prenylated compounds synthesized were found to exhibit spectrum of activity against *S. aureus* (ATCC) having MIC: $5.84 \times 10^{-3}$, $41.46 \times 10^{-2}$ and $6.19 \times 10^{-1}$ μmol/ml respectively. The compounds also displayed activity against resistance strain of *S. aureus* (SA1119B) having MIC: $5.84 \times 10^{-3}$, $7.29 \times 10^{-3}$ and $3.09 \times 10^{-1}$ μmol/ml respectively. This synthesis has been achieved and accomplished with the confirmation of it structure to that of the original natural product, thus producing the first synthesis of the natural product and providing the first synthesis of its analogs with 3-Farnesyl-2-hydroxybenzoic acid having biological activity higher than that of the original natural product.



## 1. Introduction

Resistance to antibiotics by bacterial strains especially multi-drug resistance has become a major concern in public health all over the world. Widespread use of antibacterial agents in hospitals, food production, and veterinary treatments among others has resulted in rapid emergence of antibiotics resistance. The search for new antibacterial agents, particularly those which are effective against multi-drug resistant pathogens has become a major global focus and subject of research in almost all research institutions. It is certain that most of the bacterial strains (e.g. *Staphylococcus aureus*) found in most research institutions are resistant to antibiotic. *S. aureus* is one of the most important pathogenic bacteria, causing a wide range of infections from local and usually superficial infections to extreme severe systematic ones [1]. However, mithicillin-resistance *S. aureus* (MRSA) is resistant to all β-lactam antibiotics [2] and thus making the antibiotic treatment option limited. Therefore, the widespread of multidrug resistant bacterial strains necessitates the discovery of new class of antibacterial and compounds that inhibit these resistance mechanisms [3].

Medicinal plants are used for centuries as remedies for human diseases and offer the richest biosources of drugs or traditional medical system, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entities for synthetic drugs [4]. Among the estimated 250,000–500,000 plant species [5], *Piper* species are estimated to contain more than 4000 species widely distributed in the tropical and subtropical region of the world, its species are chemically very rich and contained major classes of compounds [6,7] of which among the classes of compounds include prenylated benzoic acid derivatives [8].

3-Farnesyl-2-hydroxybenzoic acid (**1**) is an antibacterial compound isolated and characterized from the leaves of *Piper*

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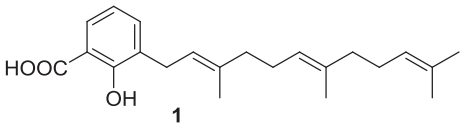
http://dx.doi.org/10.1016/j.fitote.2014.01.005

*multiplinervium*. The plant is traditionally used by the local Kuna Indians of Panama for the treatment of stomach ache. Compound **1** was found to have broad spectrum of activity against both Gram positive and Gram negative bacteria including *Escherichia coli*, *S. aureus* and *Helicobacter pylori* [9]. **1** is composed of $C_{22}H_{31}O_3$ with a molecular weight of 342.2273 and possessed a remarkable structure that the farnesyl group is found at the *ortho* position to hydroxyl group of phenol.




**1**

Phytochemical investigations previously carried out on different *Piper* species revealed to contain several interesting prenylated benzoic acid with interesting biological activities and have been described to contain structurally similar compounds [10,11]. Despite the frequent isolation of these interesting compounds, there is less report available on the antibacterial activity from *Piper* species. In view of this, the present studies aimed to synthesize a natural antibacterial compound **1** and its analogs and evaluate them against bacteria. This compound is given much interest because of the increasing demand on antibacterial agents against multi-drug resistant bacteria.

## 2. Materials and methods

All the chemical reactions were conducted inside the fume cupboard under an inert gas atmosphere of either argon or nitrogen using well oven-dried glassware. THF and ether were allowed to dry distilled over sodium before use. Most reagents and equipments used were commercially available and are obtained from Sigma Aldrich while $CO_2$ was provided by passing through a column of Drierite. All the reactions were monitored by TLC (Silica gel $60F_{254}$), and spotted under UV and the major spot identified by spraying with PMA in EtOH. The synthesized compounds and their intermediates were purified by chromatography and analyzed by NMR spectroscopy recorded on Bruker Avance spectrometer ($^1$H 400 MHz, $^{13}$C 100 MHz in $CDCl_3$, δ 7.26 as internal reference). The spectral data were recorded in ppm (δ), coupling constant in Hertz, NMR pick patterns are in broad (br), singlets (s), doublets (d), double-doublet (dd), triplets (t) and multiplets (m). Mass spectroscopy was recorded on a Finnigan MassLab Navigator quadruple instrument using electrospray (ES) visualization.

### 2.1. Synthesis of 3-Farnesyl-2-hydroxybenzoic acid (*1*) and its analogs

All the reaction procedures in this experiment were followed according to the procedure reported [12] as outlined in Fig. 1. Sodium hydride (1.5 Eq.) was suspended in tetrahydrofuran (40 mL) and allowed to cool to 0 °C under argon atmosphere. To the NaH suspension, **4** dissolved in dry THF was added dropwise and allowed to stir at r.t for 2 h. To the reaction mixture, Chloromethyl methyl ether (3 Eq.) was added dropwise and allowed to stir for 2 h. The reaction mixture was concentrated in a rotary evaporator and the residue partitioned between ether and 0.1 M NaOH. The aqueous layer was extracted again with ether and the organic layers were then combined, washed with 0.1 M NaOH and with brine respectively. The organic layers were dried over $MgSO_4$, filtered and evaporated to dryness in a rotary evaporator. The organic oil obtained was then purified by column chromatography. **5** (3.38 mmol) was dissolved in ether (5 mL) and allowed to cool to −78 °C. To the reaction mixture, *t*-BuLi (2.2 Eq) was added dropwise over 5 min and allowed to stir maintaining the temperature at −78 °C for over 1 h. To the reaction mixture, Farnesyl, geranyl or dimethylallyl bromide was added dropwise over 2 min and the reaction mixture was allowed to stir at r.t over 1 h. The reaction mixture was then diluted with ether and quenched with saturated $Na_2CO_3$ (5 mL). The aqueous layer was extracted with ether and the organic layers was then combined and washed with brine. The organic extract was dried over $MgSO_4$, filtered and evaporated to dryness in a rotary evaporator. The crude product obtained was purified by flash chromatography and further purified by TLC. **6, 7**, or **8** was dissolved in THF and allowed to cool to −78 °C. To the reaction mixture, *t*-BuLi (2.2 Eq.) was added dropwise over 5 min and allowed to stir maintaining the temperature at −78 °C for over 1.5 h. To the reaction mixture, $CO_{2(g)}$ was bubbled directly via a column of drierite and allowed to stir at −78 °C for over 1.5 h. HCl was added to the reaction mixture at r.t and allowed to stir overnight. The resulting reaction mixture was extracted twice with ether, washed with brine and dried over $MgSO_4$. The organic filtrate obtained was evaporated to dryness in a rotary evaporator and purified by preparative TLC.

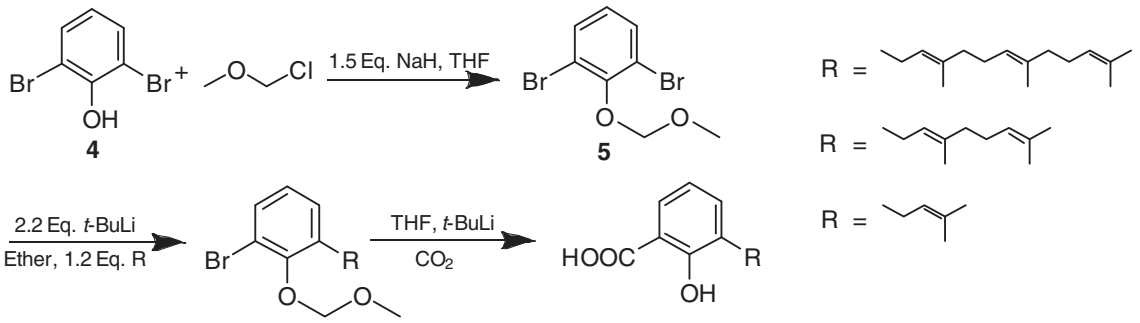



**Fig. 1.** Synthesis of 3-Farnesyl-2-hydroxybenzoic acid and it analogs in three steps.

### 2.2. Assessment of antibacterial activity

*S. aureus* (ATCC) and *S. aureus* (SA1119B) were used in the assessment of antibacterial assay. The bacterial strains used were obtained from the American Type Culture Collection (ATCC). All the compounds were determined in duplicate by minimum inhibitory concentration (MIC) values using micro-broth dilution method [13] and Norfloxacin was used as positive control. The microplate was incubated overnight at 37 °C and the MIC values were determined as the lowest concentration of the compounds that inhibits visible growth of the bacterial strains.

## 3. Result and discussion

### 3.1. Chemical synthesis

The basic principles of the reactions are highlighted in Fig. 1. The spectral data of the synthesized compounds and their intermediates are given below:

*1, 3-dibromo-2-methoxymethoxybenzene* (***5***)
Colorless oil, (12.05 g, 88%); *Rf* 0.34 (9:1 Hexane/EtOAc), $^1$H NMR (400 MHz, $CDCl_3$) δ: 3.73(s, 3H), 5.18(s, 2H), 6.88(t, $J$ = 8.0 Hz, 1H), 7.52(d, $J$ = 8.0 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 58.5, 99.6, 188.6, 125.5, 132.9, 151.9.

*2-bromo-6-Farnesyl-1-methoxymethoxybenzene* (***9***)
Compound **9** was obtained as (0.45 g, 21%); *Rf* 0.54 (19:1, hexane/EtOAc), $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.60 (s, 6H), 1.68 (s, 3H), 1.78 (s, 3H), 1.95–2.14 (m, 8H), 3.47 (d, 2H), 3.65 (s, 3H), 5.09 (s, 2H), 5.10 (m, 2H), 5.31 (m, 1H), 6.93 (t, $J$ = 8.0 Hz, 1H), 7.13 (dd, $J$ = 7.6 Hz, 1H), 7.39 (dd, $J$ = 7.6 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 16.39, 16.52, 18.04, 26.05, 26.84, 27.08, 29.10, 40.03, 58.18, 100.14, 117.71, 121.68, 121.72, 122.32, 124.35, 124.69, 124.72, 125.94, 129.42, 129.89, 131.41, 131.65, 135.52

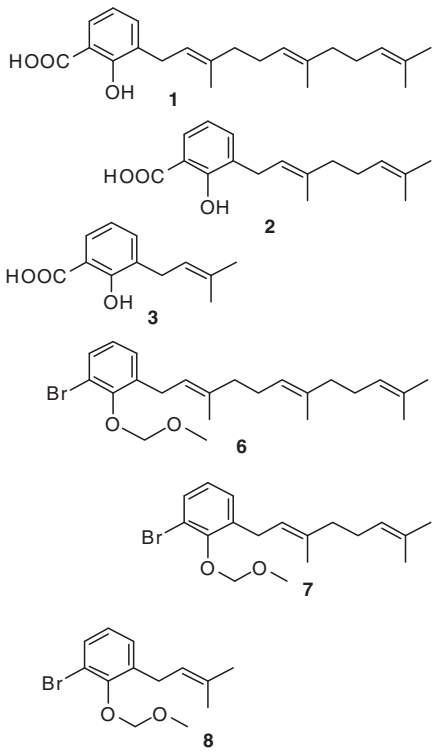



**Fig. 2.** Structure of 3-Farnesyl-2-hydroxybenzoic acid and it derivatives.

*2-bromo-6-geranyl-1-methoxymethoxybenzene* (***10***)
**10** was obtained as (0.167 g, 14%); *Rf* 0.59(9:1 hexane/EtOAc), $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.60(s, 3H), 1.68(s, 3H), 1.70(s, 3H), 2.06–2.13 (m, 4H), 3.46(d, $J$ = 7.12 Hz, 2H), 3.65(s, 3H), 5.09(s, 2H), 5.10(m, 1H), 5.30(m, $J$ = 7.2 Hz, 1H), 6.93(t, $J$ = 7.8 Hz, 1H), 7.13(dd, $J$ = 7.6 Hz, 1H), 7.40(dd, $J$ = 7.9 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 16.5, 18.1, 26.1, 40.0, 26.9, 29.1, 58.2, 100.1, 124.5, 122.4, 125.9, 129.4, 131.4, 131.9, 117.7, 153.0, 137.4, 137.8.

*2-bromo-6-dimethlyallyl-1-methoxymethoxybenzene* (***11***)
**11** was obtained as (0.120 g, 12.4%), *Rf* 0.34 (10% EtAOc/Hexane) $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.71(s, 3H), 1.75 (s, 3H3.45 (d, 2H), 3.65 (s, 3H), 5.09 (s, 2H), 5.30 (m, 1H), 6.93 (t, $J$ = 8.0 Hz, 1H), 7.12 (dd, $J$ = 6.8 Hz, 1H), 7.39 (dd, $J$ = 7.9 Hz 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 18.21, 26.08, 29.32, 58.20, 100.17, 117.75, 122.45, 125.96, 129.48, 131.45, 133.68, 137.82, 152.97.

*3-Farnesyl-2-hydroxybenzoic acid* (***1***)
**1** was afforded in 0.033 g, 10.7%; *Rf* 0.4 (3:1:2 drops $CHCl_3$/MeOH/AcOH), $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.61 (s, 6H), 1.67 (s, 3H), 1.71 (s, 3H), 1.96–2.15 (m, 8H), 3.39 (d, 2H), 5.11 (m, 2H), 5.33 (br m, 1H), 6.86 (br t, $J$ = 7.52 Hz, 1H), 7.39 (dd, $J$ = 7.12 Hz, 1H), 7.79 (dd, $J$ = 7.76 Hz, 1H), 10.67 (br, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 16.40, 16.46, 18.03, 26.03, 26.88, 27.10, 27.99, 40.10, 40.99, 111.01, 119.37, 120.79, 124.46, 124.74, 128.79, 130.69, 131.63, 135.47, 136.77, 138.20, 160.70, 175.43. Electrospray (ES) $m/z$ calculated for $C_{22}H_{31}O_3$ $(M + K)^+$ 381.24, found 342.14 and $(M + H)^-$ 341.41, found 342.42.

*3-Geranyl-2-hydroxybenzoic acid* (***2***)
**2** was obtained as pale yellow oil in 0.029 g, 22.1%; *Rf* 0.38 (3:1:2 drops $CHCl_3$/MeOH/AcOH), $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.60 (s, 3H), 1.68 (s, 3H), 1.70 (s, 3H), 2.13–2.05 (m, 4H), 3.37 (d, $J$ = 7.0 Hz, 2H), 5.11 (t, $J$ = 6.08 Hz, 1H), 5.33 (br t, $J$ = 6.8 Hz, 1H), 6.82 (br, 1H), 7.35 (d, $J$ = 7.0 Hz, 1H), 7.75 (br d, 1H). Electrospray (ES) $m/z$ calculated for $C_{17}H_{22}O_3$ $(M + H)^-$ 273.36, found 274.368.

*3-Dimethylallyl-2-hydroxybenzoic acid* (***3***)
**5** was obtained as amorphous solid in 0.0203 g, 23.3%; *Rf* 0.6 (3:1 $CHCl_3$/MeOH), $^1$H NMR (400 MHz, $CDCl_3$) δ: 1.72 (s, 3H), 1.76 (s, 3H), 3.37 (d, 2H), 5.32 (br t, $J$ = 7.16 Hz, 1H), 6.84 (br t, $J$ = 7.2 Hz, 1H), 7.36 (dd, $J$ = 7.3 Hz, 1H), 7.78 (br d, 1H). Electrospray (ES) $m/z$ calculated for $C_{12}H_{14}O_3$ $(M + H)^-$ 205.36, found 206.878.

The synthesis of prenylated salicylic acid commenced with the synthesis of the starting material 2,6-dibromo MOM protected phenol (**5**) in larger scale from commercially readily available 2,6-bromophenol (**4**). Compound **5** was successfully protected using Chloromethyl methyl ether (MOMCl) to afford the MOM ether in high yield (12.05 g, 88%) as shown in Fig. 1. The $^1$H NMR of **5** conformed to the spectral data previously reported [12] in the presence of two aromatic proton signals at δ 6.88(t, $J = 8.0$ Hz, 1H) and 7.52(d, $J = 8.0$ Hz, 2H), two singlets at δ: 3.73(s, 3H) and 5.18(s, 2H) which was assigned to $CH_3$ and $CH_2$ of MOM respectively.

The common method widely used for the prenylation of aromatic compounds is via halogen–metal exchange [14]. The desired compounds **9**, **10** and **11**, were successfully afforded in 21%, 14% and 12.4% yield respectively.

However, the remaining bromide at the *ortho* position to hydroxyl group of phenol is subsequently exchanged by bromine–lithium exchange and trapped with carbon dioxide at −78 °C. The addition of acidic workup (6 M HCl) protonated $CO_2$ and cleaved the MOM ether in situ at room temperature [15] to afford the desired compounds **1**, **2**, and **3** in 10%, 23.3% and 22.1% yield respectively. The $^1$H NMR of **1** conformed to the previously reported spectral data [9].

### 3.2. Antibacterial assessment

All the compounds **1**, **2**, and **3** synthesized were evaluated against both *S. aureus* (ATCC) and resistance *S. aureus* (SA1119B) as presented in Table 1. All the compounds showed activity against both *S. aureus* (ATCC) and the SA1119B type *S. aureus* using a standard antibacterial drug (Norfloxacin) as positive control. Compound **1** (MIC: $5.84 \times 10^{-3}$ μmol/ml) and **2** (MIC: $1.46 \times 10^{-2}$ μmol/ml) exhibit higher activity against *S. aureus* (ATCC) with **1** being the most active while compound **3** (MIC: $6.19 \times 10^{-1}$ μmol/ml) has the lowest activity. All the three compounds displayed more activity against SA1119B type than that evaluated on ATCC type. Both compounds **1** and **2** displayed activity with MIC value at $5.84 \times 10^{-3}$ and $7.29 \times 10^{-3}$ μmol/ml much higher than that of Norfloxacin (MIC: $1.57 \times 10^{-3}$ μmol/ml) while **3** has the minimum activity with MIC value at $3.09 \times 10^{-1}$ μmol/ml. Compound **1**, however, displayed higher activity against *S. aureus* (ATCC) at MIC: $5.84 \times 10^{-3}$ μmol/ml in comparison to that reported natural product **1** (MIC: 12.5 μg/ml) [9].

Bacterial resistance modulators are compounds which potentiate the activity of an antibiotic against a resistant strain. These compounds may target a resistance mechanism such as the inhibition of multidrug resistance (MDR), e.g. inhibition of the NorA MDR pump of *S. aureus* effluxes [3,16]. However, few plant secondary metabolites found as prenylated, geranylated or farnesylated have been reported to inhibit NorA MDR pump of *S. aureus* effluxes. Apart from prenylated benzoic acid derivative reported with antibacterial activity from *P. multiplinervium* [9], only few plant polyphenolic compounds found as prenylated, geranylated or farnesylated have been reported to show antibacterial activity or inhibition of NorA MDR pump of *S. aureus* effluxes. Iinuma et. al [17] reported the antibacterial activity of rubraxanthone isolated from the bark of *Garcinia dioica* Dl. with activity against both methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus*. Rubraxanthone showed greater activity than that of the antibiotic Vancomycin against staphylococcal strains having MIC values ranging from 0.31 to 1.25 μg/ml. In another report, 2 prenylated derivatives of benzoic acid 4-hydroxy-(3′,7′-dimethyl-1′-oxo-octa-E-2′-6′-dienyl) benzoic acid and 4-hydroxy-(3′,7′-dimethyl-1′-oxo-octa-2′-Z-6′-dienyl) benzoic acid isolated from *Piper gaudichaudianum* showed antibacterial activity against *S. aureus* and *Baccilus subtilis* having MIC of 31.25 μg/mL and 12.5 μg/mL for *S. aureus*, respectively, and 7.81 μg/ml and 6.25 μg/ml for *B. subtilis*, respectively [2]. Prenylated flavonoids with activity against methicillin-resistant *S. aureus* have also been reported in *Delia scandens* val. Paucifolia [18]. One of the prenylated flavonoids isolated from the root of this plant is 2(S)-5′-(-1‴,1‴-dimethylallyl)-8-(3″,3″-dimethylallyl)-2′,4′,5,7-tetrahydroxyflavanone. This compound displayed spectrum of activity against both resistant and sensitive strains of *S. aureus* having MIC value of 1.56 μg/ml. It is noteworthy to suggest that the isoprenyl side chain on the salicylic acid of these compounds plays a crucial role in the antibacterial activity.

The literature survey revealed that there is no report so far on compounds **2** and **3** and their antibacterial properties. Therefore, this research reports the synthesis and antibacterial evaluation of two new compounds **1** and **2**. The present studies showed that the antibacterial activity is higher in longer isoprenyl side chain on the salicylic acid than those of the shorter isoprenyl side chain. The results show that the longer the side chain the greater the biological activity, which might result from an enhanced ability to cross the bacterial lipid membrane.

**Table 1**
Antibacterial activity of compounds **1**, **2**, and **3**.

| Compound | *S. aureus* (ATTC) MIC (μmol/ml) | *S. aureus* (SA1119B) MIC (μmol/ml) |
|---|---|---|
| **1** | $5.84 \times 10^{-3}$ | $5.84 \times 10^{-3}$ |
| **2** | $1.46 \times 10^{-2}$ | $7.29 \times 10^{-3}$ |
| **3** | $6.19 \times 10^{-1}$ | $3.09 \times 10^{-1}$ |
| Norfloxacin | $1.57 \times 10^{-3}$ | $1.00 \times 10^{-1}$ |

MIC: minimal inhibitory concentration (μmol/ml).

## 4. Conclusion

The search for potential antibacterial agent especially against multi-drug resistance bacteria led to the synthesis of prenylated salicylic acid isolated from *P. multiplinervium*. However, the present research studies have been a success in the synthesis of natural 3-Farnesyl-2-hydroxybenzoic acid. Additionally, two new prenylated compounds **2** and **3** have been successfully synthesized. All compounds synthesized has been achieved and accomplished with the confirmation of its structure to that of the original natural product, thus producing the first synthesis of the natural product and providing the first synthesis of its analogs. All the three prenylated compounds displayed spectrum of activity against both *S. aureus* (ATCC) and *S. aureus* (SA1119B).

## 5. Conflict on interest

Authors declare that there are no conflict of interest.

## Acknowledgments

This research project was carried out in part fulfillment of the requirements for the Master in Pharmacognosy degree, UCL School of Pharmacy.

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