

## CHEMICAL INVESTIGATION OF SECONDARY METABOLITES FROM *MAGNOLIA GRANDIFLORA*

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The methylene chloride-methanol (1:1) extract of the air-dried aerial parts of *Magnolia grandiflora* was fractionated and separated to obtain the isolated compounds by different chromatographic techniques. Structures of the isolated compounds were determined on the basis of the extensive spectroscopic analysis, including 1D and 2D NMR and compared with the literature data. Three secondary metabolites were isolated from *Magnolia grandiflora* in this study. Of which two sesquiterpenoidal compounds; parthenolide (1) and 1,10-epoxy-parthenolide (2), together with an aromatic compound identified as three-4-hydroxyphenyl propane-7,8-diol, 7-O-ethylether (3), which firstly isolated from the genus *Magnolia*. Our study aims to further phytochemical investigation of *M. grandiflora* to discover more secondary metabolites characterized with wide range of therapeutic values.

**Keywords:** *Magnolia grandiflora*, Magnoliaceae, Sesquiterpenes, Aromatic.

### INTRODUCTION

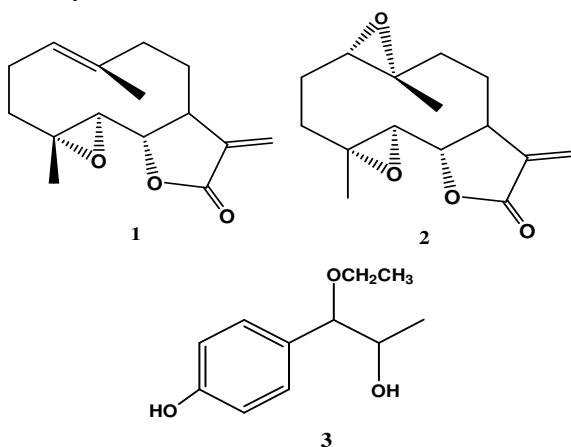
The genus *Magnolia*, belonging to the family Magnoliaceae, Tree or shrubs, composed of approximately 240 species widely distributed in through tropical and temperate regions, mainly in China, India, Japan and Malaysia<sup>1</sup>.

Many species of *Magnolia* have great therapeutic values and widely used in Chinese traditional medicines for treatment wide range of diseases. The flower buds of *Magnolia denudate* and *Magnolia sprengeri* are currently used to treat sinusitis, nasal congestion, empyema and allergic rhinitis<sup>2</sup>. Also, many diseases, such as lung disorders, cough, asthma, edema and intestinal disorders were treated by using the bark of *Magnolia officinalis*<sup>3</sup>. The previous phytochemical studies on genus *Magnolia* have been revealed the presence of alkaloids<sup>4</sup>, lignans<sup>5&6</sup>, and sesquiterpenoids<sup>7&8</sup>.

*Magnolia grandiflora*, (Magnoliaceae), a large evergreen tree, is native to north America and also, cultivated in South of Yangtze river basin<sup>9</sup>. The plant of *M. grandiflora* is commonly used traditionally in folk medicine in treatment of GIT disorders, heat disturbances, and rheumatic arthritis, beside its value as ornamental plant<sup>10</sup>. Several secondary metabolites have been reported from *M. grandiflora* including sesquiterpenes<sup>11-14</sup>, biphenyls<sup>15</sup>, alkaloids<sup>16</sup>, and Lignan glycosides<sup>17</sup>.

Sesquiterpenes lactones are considered the most important secondary metabolites of different members of families as Asteraceae, Acanthaceae, Apiaceae and Magnoliaceae. They are characterized with vast array of chemical structures and diversity of biological activities including; anti-inflammatory, antihyperalgesic effects, cytotoxicity insect antifeedant, plant growth regulating, antibacterial, antifungal and antitumorigenic

properties<sup>7,18-23</sup>. However, a few sesquiterpenes lactones were reported from *M. grandiflora*. Therefore, further phytochemical studies on this species are required to find more potential bioactive compounds. As a result, two sesquiterpenes lactones (**1-2**), and an aromatic compound (**3**), were isolated from the methylene chloride/methanol (1:1 v/v) extract of the leaves of *M. grandiflora* in the current study.



**Fig. 1:** The isolated compounds from *M. grandiflora*.

## MATERIALS AND METHODS

General experimental procedure <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub> and CD<sub>3</sub>OD), <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub> and CD<sub>3</sub>OD) and the 2D spectra (<sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC, and NOSY) were recorded on the JEOLEAC 600 MHz spectrometer, with tetramethylsilane (TMS) as an internal standard. Electron impact mass spectrometry (EI-MS) analyses were recorded on a JEOLSX102A mass spectrometer. Column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh) and octadecylsilyl (ODS) (Pharmacia Co. Tokyo, Japan). TLC was performed on silica gel 60 F<sub>254</sub> plated (0.25 mm, Merck Co.), and spots were detected under UV light and colored by spraying with 10% H<sub>2</sub>SO<sub>4</sub> solution followed by heating.

### Plant material

The leaves of *M. grandiflora* were collected from tree cultivated in private gardens near Mansoura, Egypt, on March, 2018. The plant was kindly identified and authenticated by Prof. Dr. Mona M. Marzouk,

Professor of Taxonomy, National Research Center, Cairo, Egypt. A voucher specimen has been deposited in Department of Chemistry, Faculty of Sciences, El-Minia University (No. 300-2018). The plant material was air-dried, powdered and kept in closed containers.

### Extraction and isolation

The dried leaves (0.5 kg) of *M. grandiflora* were powdered and extracted with CH<sub>2</sub>Cl<sub>2</sub> : MeOH (1:1 v/v) at room temperature. The extract was concentrated *in vacuo* to obtain a gummy residue (40 g). The concentrated crude extract was fractionated on silica gel Column chromatography (200 g silica, 5 × 60 cm). Elution was performed starting with *n*-hexane (3L) and the polarity is gradually increased with ethyl acetate (25% increments) till 100% ethyl acetate (2L, each). Fractions (500 ml, each) were collected and monitored by TLC using three solvent systems, CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 7.5:0.5 v/v, *n*-hexane-ethyl acetate, 4:1 v/v and *n*-hexane-ethyl acetate, 3:1 v/v. Vanillin-sulphuric acid spray reagent was used for compound spots detection. Similar fractions were pooled according to their chromatographic properties to yield six collected fractions as the following: A (8.0 g), B (3.5 g), C (7.0 g), D (6.0 g), E (5.0 g), and F (7.5 g). TLC investigations of these six fractions showed that fractions C, D, E and F exhibited promising chromatographic profiles compared to other fractions. Therefore, these fractions were selected for further chromatographic processing. Fractions C, D, and E were separately fractionated by reversed phase chromatography on octadecylsilyl (ODS) column (7.0, 6.0 and 5.0 g, respectively, 3 × 90 cm). Elution was started with 80:20 v/v% (methanol : H<sub>2</sub>O) till 100% to give 3 subfractions for each fraction. Subfractions C-1 (3.0 g), D-2 (4.5 g), E-1 (3.5 g) were then subjected to preparative separation on reversed phase HPLC. Subfraction C-1 yielded compound **1** (10 mg). Subfraction D-2 yielded compound **2** (6 mg). Subfraction E-1 yielded compounds **3** (8 mg). Compounds (**1-2**) showed sesquiterpenoids basic skeleton, however compound **3** was an aromatic compound according to their 1D and 2D NMR data (Fig. 1).

## RESULTS AND DISCUSSION

### Parthenolide (1)

Colourless crystal;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.21 (1H, dd,  $J= 11.6, 2.4$  Hz, H-1), 2.35-2.48 (1H, m, H-2a), 2.11-2.24 (1H, m, H-2b), 1.25 (2H, m, H-3), 2.11-2.24 (1H, m, H-3b), 2.80 (1H, d,  $J= 8.5$  Hz, H-5), 3.88 (1H, dd,  $J= 12, 9.2, 8.5$  Hz, H-6), 2.78 (1H, m, H-7), 1.74 (1H, m, H-8a), 2.11-2.24 (1H, m, H-8b), 2.11-2.24 (1H, m, H-9a), 2.35-2.48 (1H, m, H-9b), 5.61 (1H, d,  $J= 3.0$  Hz, H-13a), 6.25 (1H, d,  $J= 3.5$  Hz, H-13b), 1.72 (1H, s, H-14), 1.31 (1H, s, H-15);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$ : 125.1 (C-1), 24.1 (C-2), 36.3 (C-3), 61.5 (C-4), 66.3 (C-5), 82.4 (C-6), 47.6 (C-7), 30.6 (C-8), 41.1 (C-9), 134.6 (C-10), 139.2 (C-11), 169.2 (C-12), 121.1 (C-13), 16.9 (C-14), 17.2 (C-15); EI-MS  $m/z$  248  $[\text{M}]^+$ ,  $\text{C}_{15}\text{H}_{20}\text{O}_3$ .

### 1,10-Epoxy parthenolide (2)

Colourless crystal;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$ : 2.81 (1H, dd,  $J= 10.6$  &  $2.4$  Hz, H-1), 2.35-2.48 (1H, m, H-2a), 2.11-2.24 (1H, m, H-2b), 1.25 (2H, m, H-3), 2.11-2.24 (1H, m, H-3b), 2.86 (1H, d,  $J= 8.5$  Hz, H-5), 3.86 (1H, dd,  $J= 12, 9.2, 8.5$  Hz, H-6), 2.78 (1H, m, H-7), 1.74 (1H, m, H-8a), 2.11-2.24 (1H, m, H-8b), 2.11-2.24 (1H, m, H-9a), 2.35-2.48 (1H, m, H-9b), 5.61 (1H, d,  $J= 3.0$  Hz, H-13a), 6.25 (1H, d,  $J= 3.5$  Hz, H-13b), 1.28 (1H, s, H-14), 1.31 (1H, s, H-15);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$ : 63.2 (C-1), 24.0 (C-2), 36.2 (C-3), 61.5 (C-4), 66.4 (C-5), 82.3 (C-6), 47.5 (C-7), 30.5 (C-8), 41.0 (C-9), 62.0 (C-10), 139.2 (C-11), 169.2 (C-12), 121.0 (C-13), 16.8 (C-14), 17.1 (C-15); EI-MS  $m/z$  264  $[\text{M}]^+$ ,  $\text{C}_{15}\text{H}_{20}\text{O}_4$ .

### Threo-4-hydroxyphenyl propane-7,8-diol, 7-O-ethylether (3)

White yellowish oil;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$ : 3.31 (1H, dd,  $J= 11, 9.5$  Hz, H-1), 1.63 (1H, m, H-2a), 2.01 (1H, m, H-2b), 1.60 (1H, m, H-3a), 1.27 (1H, m, H-5), 0.48 (1H, dd,  $J= 12, 9$  Hz, H-6), 0.65 (1H, ddd,  $J= 10, 9, 6.5$  Hz, H-7), 2.15 (1H, m, H-8a), 1.27 (1H, m, H-8b), 2.45 (1H, dd,  $J= 10, 6.5$  Hz, H-9a), 2.21 (1H, dd,  $J= 10, 6.5$  Hz, H-9b), 1.14 (3H, s, H-12), 1.12 (3H, s, H-13), 4.68 (1H, br.s, H-14a), 4.85 (1H, br.s, H-14b);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$ : 53.4 (C-1), 25.0 (C-2), 41.9 (C-3), 81.1 (C-4), 54.4 (C-5), 30.0 (C-6), 25.5 (C-7), 38.5 (C-8), 26.5 (C-9), 153.5 (C-10), 20.1 (C-11), 16.2 (C-12), 28.5 (C-13), 106.3 (C-14), 27.5 (C-15); EI-MS  $m/z$  196  $[\text{M}]^+$ ,  $\text{C}_{11}\text{H}_{16}\text{O}_3$ .

### Identification of purified compounds

The  $\text{CH}_2\text{Cl}_2$ -MeOH (1:1) extract of the leaves of *M. grandiflora* were fractionated on flash silica gel CC and these fractions were subjected separately to further fractionation by reversed phase chromatography on octadecylsilyl (ODS) column to obtain number of subfractions. The promising subfractions were isolated and purified by reversed phase HPLC resulted in the isolation of nine secondary metabolites (1-3) (Fig. 1). Compounds (1-2) were identified as parthenolide (1), previously isolated from *Magnolia champaca*<sup>24</sup>, 1,10-epoxy parthenolide (2), also previously reported from *M. grandiflora*<sup>25</sup> besides, an aromatic compound was identified as; threo-4-hydroxyphenyl propane-7,8-diol, 7-O-ethylether (3), previously isolated from *Narvalina domingensis*<sup>26</sup>. As far as could be ascertained, this is the first report of compound (3) from genus *Magnolia*. The structures of isolated compounds were elucidated by using different spectroscopic analysis;  $^1\text{H}$  NMR,  $^1\text{H}$ - $^1\text{H}$  COSY,  $^{13}\text{C}$  NMR, DEPT, HMQC, HMBC and EI-MS, as well as by comparison with reported data. The presence of parthenolide (1) and 1,10-epoxy parthenolide (2) as sesquiterpene lactones isolated from *M. grandiflora*, may introduce a scientific evidence that supports the traditional uses of plant in the treatment of GIT disorders, rheumatic arthritis, sinusitis, nasal congestion, empyema and allergic rhinitis. Therefore, the isolated compounds should be subjected to further studies to assessment their biological activities to support the traditional uses of plant, validate their use, facilitate their pharmaceutical formulation and determine their mode of action. Also, the previous study recommend the intensive propagation of indigenous species of *Magnolia* especially *M. grandiflora* to search for more bioactive components and may be used in the pharmaceutical industry.

### Conclusion

Three secondary metabolites (1-3) were characterized from the  $\text{CH}_2\text{Cl}_2$ /MeOH (1:1 v/v) extract of the leaves of *M. grandiflora*, including two sesquiterpene lactones (1-2) and a simple aromatic compound (3). Compounds

(3) was firstly isolated from genus *Magnolia*. The results of the current study may introduce a scientifically support the traditional uses of the plant in the treatment of GIT disorders, rheumatic arthritis, and other diseases, due to the wide therapeutic actions of sesquiterpenes lactones content of *M. grandiflora*. Further phytochemical investigation and biological evaluations on *Magnolia* species especially *M. grandiflora* are required for discovery new bioactive secondary metabolites that may be used as phytotherapeutic agents in treatment of different diseases.

#### Conflict of interests

Declared none.

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## فحص كيميائي للمركبات الثانوية من نبات ماجنوليا جراندفلورا

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تم استخلاص الأجزاء الهوائية لنبات الماجنوليا جراندفلورا *Magnolia grandiflora* بواسطة مزيج من الكحول الميثيلي وكلوريد الميثيلين بنسبة (1:1) ثم تمت تجزئته وفصل المستخلص الكلي وذلك للحصول علي المركبات النقية المفصولة باستخدام طرق الفصل الكروماتوجرافية المختلفة. تم تعريف التركيب الكيميائي للمركبات المفصولة بواسطة تحاليل طيف الرنين النووي المغناطيسي ذات البعد الواحد وثنائي البعد والطرق الطيفية الاخرى وبالمقارنة مع المركبات المفصولة سابقا عن طريق المسح المرجعي. حيث تم فصل وتعريف 3 مركبات في صورة نقيه ، منهم مركبين من مركبات السيسكوترابين أحدهما يسمى (1) Parthenolide والمركب الثاني يسمى (2) 1,10-epoxy parthenolide بينما كان المركب الثالث عبارة عن مركب اروماتي يسمى threo-4-hydroxyphenyl propane-7,8-diol، والذي تم فصله لأول مره من جنس الماجنوليا *Magnolia*. تهدف هذه الدراسة الي دراسة المحتوي الكيميائي للنبات ومحاولة اكتشاف مركبات ثانوية فعالة تتميز بمدي واسع من القيمة والاهمية الطبية.