Analysis of Chemical Constituents from *Sesbania grandiflora* (L.) Poir. Leaves by LC-MS/MS

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Abstract

The selected plant *Sesbania grandiflora* (L.) Poir. (Pauk-pan-phyu) belonging to the family Fabaceae. These samples were collected from East Dagon Township, Yangon Region. It is a small tree, perennial native to tropical Asia and closely related to the Australian. The all parts of *Sesbania grandiflora* (L.) Poir. are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, leaves, flowers and fruit. The powdered samples of leaves were extracted by using two solvents including ethanol and distilled water. The two extracts were dried by freeze drier at (-60ºC). And then, unbiased metabolomics analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters, Milford, USA). According to LC-MS/MS analysis, 24 possible compounds and 25 possible compounds were obtained from positive and negative ion modes from *Sesbania grandiflora* (L.) Poir. leaves.

Key words: morphological, metabolomics, ultra-performance liquid chromatography

Introduction

Sesbania grandiflora (L.) Poir., is a soft wooded tree belonging to the family Fabaceae, commonly known as Agati is a widely available fast growing plant. It is a small tree, perennial native to tropical Asia and closely related to the Australian. It is cultivated as an ornamental plant, grows wild in hedges and shady forests (Gutteridge and Shelton, 1994).

The parts of these plants serve as a natural antioxidant. The juice of the leaves is considered to possess anthelmintic property and is used to treat worms, fever, gout, itchiness and leprosy. The flowers and young leaves of *Sesbania grandiflora* are used to supplement meals. The dried leaves of *Sesbania grandiflora* are used in some countries as a tea which is considered to have antibiotic, anthelmintic anti-tumour and contraceptive properties (Sreelatha *et al*., 2011).

Sesbania grandiflora has unique medicnal properties and used as an herbal drug for its antibiotic, anthelmintic, antitumour and contraceptive properties (Okonogi *et al*., 2007). Besides being used as food, *S. glandiflora* is also used as traditional medicine, it used to cure diarrhea, snakebite, malaria, smallpox, fever, scabies, ulcers, and gastric disorders (Bhalke *et al*., 2016). The leaves *S. glandiflora* as traditional medicine are used for antioxidant, antidiarrheal and antimicrobial activity. The utilization is related to its bioactivity, comprising hypolipidemic, wound healing, anti-ulcer, antioxidant, hepatoprotective, antidiabetic, antioxidant, antipyretic, and expectorant (Samira *et al*., 2017).

Qualitative compounds were screened in *Sesbania grandiflora*, carbohydrates, terpenoids, steroids, tannins, saponins, flavonoids, quinonines, polyphenols, cyanidin and glucoside are present (Kale *et al*., 2015). The chemical constituents observed in these plants are galactommannan, linoleic acids, beta-sitosteral, and carbohydrate. It is not only a native economic tree, but also its bark, leaves and flower can be used in traditional medicine for treatment of cold, fever, stomach disorder, diarrhea and

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jaundice and as skin cleanser (Samira *et al*., 2017). Hence, the medicinally and nutritionally important were used for the metabolomic activities in the present study. The aim and objectives are to extract the sample by using ethanol and distilled water and to determine the chemical compositions of *S. grandiflora* (L.) Poir. Leaves.

Materials and Methods

Preparation of plant materials from *Sesbania grandiflora* (L.) Poir. Leaves

The fresh samples *Sesbania grandiflora* (L.) Poir. leaves were collected from East Dagon Township, Yangon Region. The leaves were washed with tap water for any contaminants, dried thoroughly under shade and powered finely. The powder sample was stored in an air tight container for the chemical study. The extraction of compounds was carried out at the Department of Oriental Herb Science, Jonbuk University, Iksan in Korea.

Extraction of *Sesbania grandiflora* (L.) Poir. Leaves

Each 100g of leaves powder sample in *Sesbania grandiflora* (L.) Poir. was extracted using different solvents including 99% of ethanol and distilled water. The samples were soaked in ethanol for 12 hours and distilled water that was boiled in water bath (60˚C) for one hour. The two extracts were filtered through a sheet of filter paper (Whatman No. 1) and the filtrates were re-filtered through a 0.45 μm nylon membrane filter (GE Healthcare UK). The collected filtrated were dried in different processes. The ethanol extract was concentrated using a rotary evaporator with water bath at (60˚C) and the aqueous extract was concentrated using a rotary evaporator with water bath at (80˚C - 90˚C) and the two extracts were dried by freeze drier at (-60˚C). And then, the chemical constituents of *Sesbania grandiflora* (L.) Poir. leaves were analyzed by LC-MS/MS.

LC-MS/MS analysis

Unbiased metabolomics analysis was investigated using an ultra-performance liquid chromatography (UPLC) system (Waters, Milford, USA). The chromatographic separation was carried out using an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm, Waters) with a column temperature of 40° C and a flow rate of 0.5 ml/min, where the mobile phase contained solvent A (water + 0.1% formic acid) and solvent B (acetonitrile +0.1% formic acid). Metabolites were eluted using the following gradient elution conditions: 97% phase A for 0–5 min; 3–100% liner gradient phase B for 5 ~16 min; 100% phase B for 16–17 min; 100–3% reverse liner gradient phase B for 17~19 min; 97% Phase A for 19–25 min. The loading volume of each sample was 5 μl. The metabolites eluted from the column were detected by a high-resolution tandem mass spectrometer SYNAPT G2 Si HDMS QTOF (Waters) in positive and negative ion modes. The capillary voltage and the cone voltage were set at 2 kV and 40 V for positive ion mode and 1 kV and 40 V for negative ion mode respectively. The mass spectrometry data to collect by using centroid MS^E mode. The primary scan ranged from 50 to 1200 Da and the scanning time was 0.2 s. the all parent ions were fragmented using 20–40 eV. The resulting of all fragments was collected and the time was 0.2 s. In the data acquisition process, the LE signal was gained every 3 s for real-time quality correction. For accurate mass acquisition, leucine enkephalin at a flow rate of 10 μl min^{-1} was used as a lock mass by a lock spray interface to monitor the positive ($[M + H]^+$ = 556.2771) and the negative ($[M - H]^-$ = 554.2615) ion modes. Waters UNIFI V1.71 software was controlled data acquisition and analysis. MS and MS/MS modes of the scan rang were over a range of 50–1200 m/z.

Results

Morphlogical characteristics

Scientific Name - *Sesbania grandiflora* (L.) Poir.
English Name - Agati
Myanmar Name - Pauk-pan-phyu
Family - Fabaceae

Small tree, perennial. Leaves alternate, pinnately compound. Inflorescences axillary raceme. Flower complete, bisexual, irregular, zygomorphic, pentamerous, cyclic and hypogynous. Calyx (5), synsepalous, campanulate, ascending imbricate, sepaloid, bilabiate (the two upper and three lower united). Corolla 1+2+(2), apopetalous, papilionaceous, consisting of a large posterior petal, standard 1, 2 lateral petals wings and 2 posterior petals fused to form the keel, descending imbricate, petaloid (white). Stamen 5+5, dithecous, introse, dorsifixed, filaments 5 long and 5 short, longitudinal dehiscence. Gynoecium (1), monocapellary, unilocular, style long, stigma globose, ovary superior, marginal placentation. Fruits are legume. Seed endospermic.

Habit Leaves Inflorescence Flowers Fruits

Fig. 1 Morphological characters of *Sesbania grandiflora* (L.) Poir.

Chemical Constituents from *Sesbania grandiflora* (L.) Poir. Leaves

Table 1. Itemname: Sesbania ES-, Sample position: 1:A, 6, Replicate number: 1

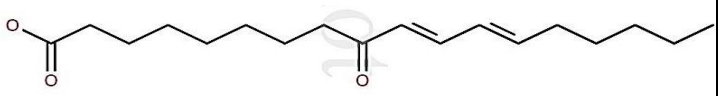
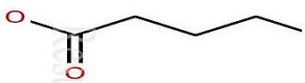
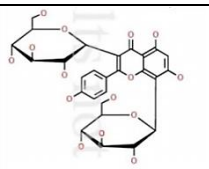
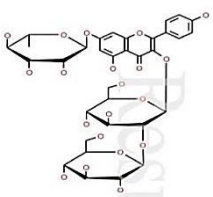
No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
1.	(E,E)-9-Oxooctadeca-10,12-dienoic acid 	294.2195	294.2196
2.	(E,E)-9-Oxooctadeca-10,12-dienoic acid 	294.2195	294.2199
3.	3,8-Di-C-glucosylapigenin 	594.1585	594.1583
4.	3-O-[β-D-Glucopyra-nosyl-(1→2)]-β-D-glucopyranosyl-7-O-α-Lglucopyranosyl-kaempferol 	756.2113	756.2125

Table 1 Contd.

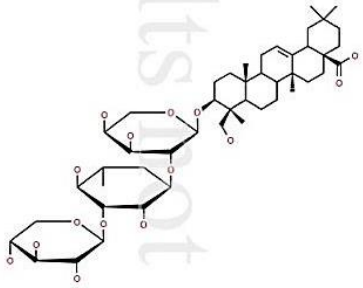
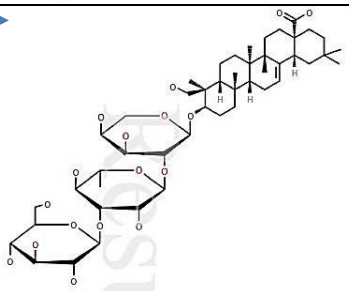
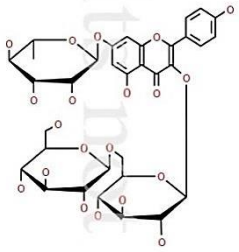
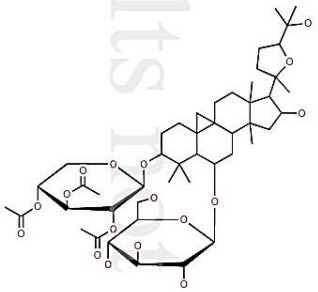
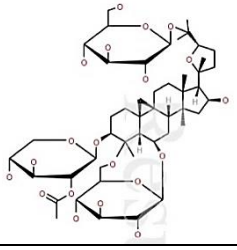
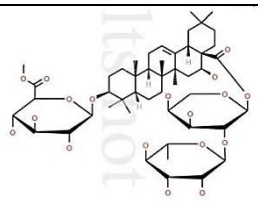
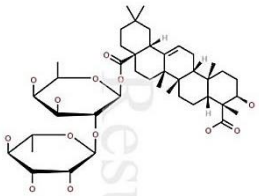
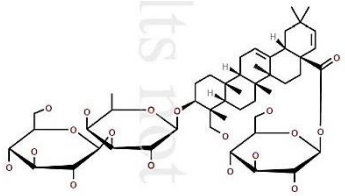
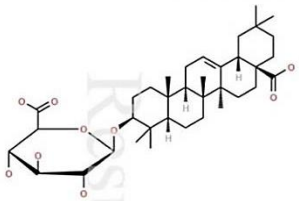
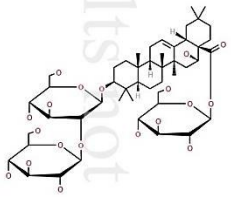
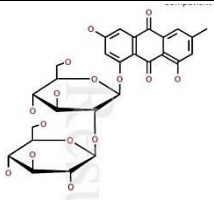
No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
5.	3-O-[β-D-Xylopyrano-syl-(1→3)-α-L-rhamno-pyranosyl-(1→2)-α-Larabinpyranosyl]-hederagenin 	866.5028	866.5015
6.	3-O-β-D-Glucopyranosyl(1 3) -α-L-rhamnopyranosyl-(1→2)-α-Larabinopyranosyl Hederagenin 	912.5083	912.5081
7.	7-α-L-Rhamnosyl kaempferol 3-O-β-D-glucopyranosyl (1→6)-β-Dglucopyranoside 	756.2113	756.2123
8.	9,12,15-Octadecatrienoic acid 	278.2246	278.2246
9.	Acetylastragaloside I 	910.4926	910.491
10.	Agroastragaloside Ⅳ 	988.5243	988.5231

Table 1 Contd.

No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
11.	Aster saponin Hb 	940.5032	940.5013
12.	Aster saponin Hb	940.5032	940.5013
13.	Aster saponin Hb	940.5032	940.5002
14.	Celosin C 	778.4503	778.4494
15.	Celosin C	778.4503	778.4493
16.	Celosin C	778.4503	778.4496
17.	Clinopodiside C 	940.5032	940.5011
18.	Deglucose chikusetsusaponin Ⅳa 	632.3924	632.3912
19.	Eclalbasaponin Ⅲ 	876.4177	876.4153
20.	Emodin-8-O-sophoroside 	594.1585	594.1583

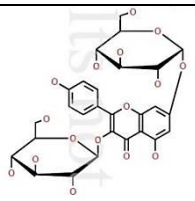
21.	Kaempferol-3,7-diglucoside_1 	610.1534	610.1542

Table 1 Contd.

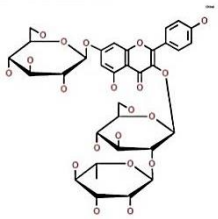
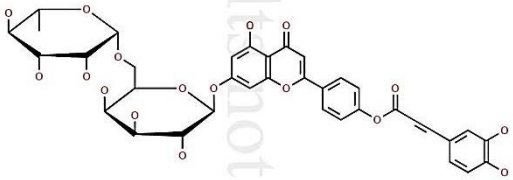
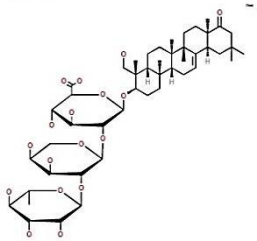
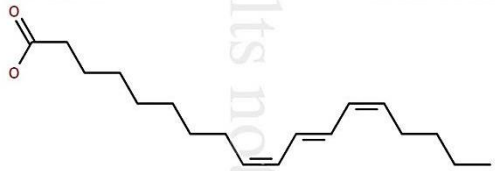
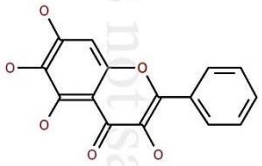
No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
22.	Kaempferol-3-rutinoside-7-glucoside 	756.2113	756.2122
23.	Menthoside 	740.1952	740.1954
24.	Soyasaponin βg 	910.4926	910.4893
25.	Trichosanic acid 	278.2246	278.2238

Table 2. Item name: Sesbania ES+, Sample position: 1:A,6, Replicate number: 1

No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
1.	3-Hydroxy baicalein 	286.0477	286.0472

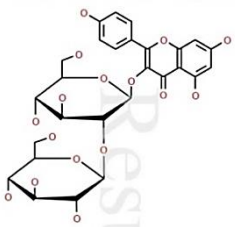
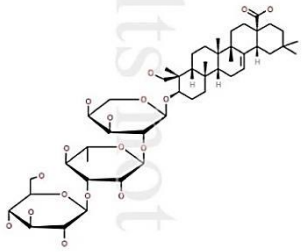
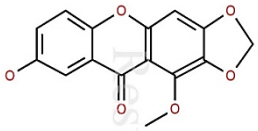
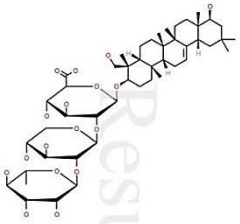
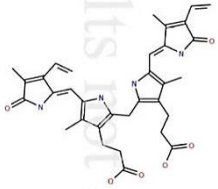
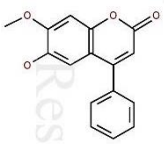
2.	3-O-[β-D-Glucopyra-nosyl-(1 2)]-β-D-glucopyranosyl-kaempferol 	610.1534	610.1531

Table 2 Contd.

No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
3.	3-O-β-D-Glucopyranosyl(1 3) -α-L-rhamnopyranosyl-(1 2) -α-Larabinopyranosyl hederagenin 	912.5083	912.5089
4.	7-Hydroxy-1-methoxy-2-methoxyxanthone 	286.0477	286.0474
5.	7-Hydroxy-1-methoxy-2-methoxyxanthone	286.0477	286.0472
6.	Astragaloside Ⅷ 	912.5083	912.5089
7.	Bilirubin 	584.2635	584.2655
8.	Bilirubin	584.2635	584.2656
9.	Bilirubin	584.2635	584.2654
10.	Dalbergin 	268.0736	268.0731

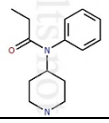
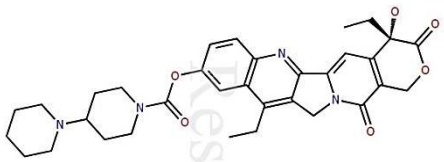
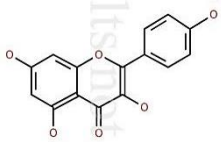
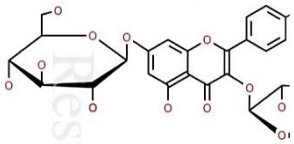
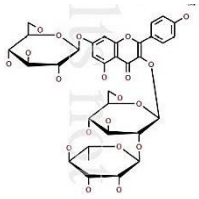
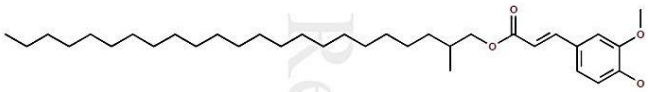
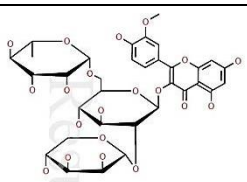
11.	Fentanyl, Nor 	232.1576	232.1569
12.	Irinotecan Chiral 	586.2791	586.2811

Table 2 Contd.

No.	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
13.	Irinotecan Chiral	586.2791	586.281
14.	Irinotecan Chiral	586.2791	586.2813
15.	Kaempferol 	286.0477	286.0473
16.	Kaempferol-3,7-di-O-β-D-glucopyranoside 	610.1534	610.1531
17.	Kaempferol-3-rutinoside-7-glucoside Chiral 	756.2113	756.2108
18.	Lignoceryl ferulate 	530.4335	530.4357
19.	Lignoceryl ferulate	530.4335	530.4357
20.	Nelumboroside B 	756.2113	756.2107
21.	Pyrophaeophorbide A	534.2631	534.2626

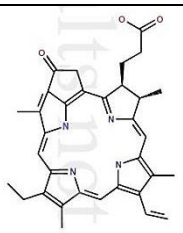
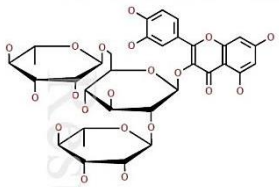
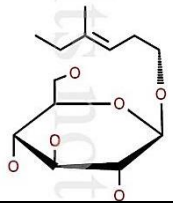
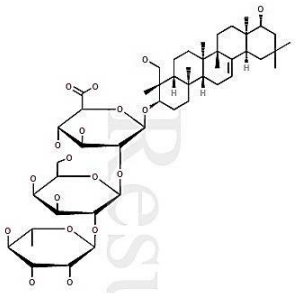
			
22.	Quercetin-3-O-(2G-α-L-rhamnosyl)-rutinoside 	756.2113	756.2108

Table 2 Contd.

No.	**Component name and Structure**	**Neutral mass (Da)**	**Observed neutral mass (Da)**
23.	Sarmentosin 	276.1573	276.1579
24.	Soyasaponin I 	942.5188	942.5189

Discussion and Conclusion

The samples of *Sesbania grandiflora* (L.) Poir. belonging to the family Fabaceae, collected from East Dagon Township, Yangon Region. The morphological and preliminary phytochemical tests were conducted at the Department of Botany, Dagon University. The various steps extraction of compounds were carried out at the Department of Oriental Herb Science, Jonbuk National University, Iksan in Korea. In the present study, the analysis of chemical constituents from *Sesbania grandiflora* (L.) Poir. leaves by LC-MS/MS were described. In the previous study, the main constituents of *Sesbania grandiflora* (L.) Poir. leaves were found to alkaloids, glycoside, reducing sugar, α-amino acids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids and starch respectively. According to LC-MS/MS analysis, 24 possible compounds and 25 possible compounds were obtained from positive and negative ion modes. In this results, *Sesbania grandiflora* (L.) Poir. leaves plant data and LC-MS/MS data were not being identical. So, these compounds

were not definitely identified by LC-MS/MS but these compounds may be checked by NMR.

Acknowledgements

My special thanks to Dr. Soe Soe Aye (Rector), Dr. Khin Moe Moe (Prorector) and Dr Htake Tin Han (Prorector), Hpa-an University for their kind permission to research paper. I also greatly indebted to Dr. Ohnmar Thein (Professor and Head) and Dr Si Si Thein (Professor), Department of Botany, Hpa-an University for their allowing submitting Fourth Myanmar-Korea Conference. I would like to express my profound gratitude to Professor Bang Keuk Soo, Project manager (JBNU) for guidance and providing infrastructural support to carry out the research work. I also wish to indebted their sincere thanks to Fourth Myanmar- Korea Conference for their allowing submitting it this article.

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