Analysis of Chemical constituents from *Acacia concinna* (Willd.) DC. Fruits by LC-MS/MS

Sandar Aung [1], Tin Tin Hla[2], Myat Myat Moe [3]

Abstract

The selected plant *Acacia concinna* (Willd.) DC. fruits (Kin-Mon- Thee) belongs to the family Mimosaceae. It is a climbing shrub native to Asia, common in the warm plains of central and south India. It is one of the Ayurvedic medicinal plants. The plant parts are used for the dry powder or the extracts are the barks, leaves or pods. The powdered sample of fruits were extracted by using two solvents including ethanol and distilled water. The two extracts were dried by freeze drier at (-62□ C). And then, unbiased metabolomics analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters, Milford, USA). According to LC-MS/MS analysis,19 possible compounds and 12 possible compounds were obtained from positive and negative ion modes from *Acacia concinna* (Willd.) DC. fruits.

Key Words: Ayurvedic, metabolomics, ultra-performance liquid chromatography

Introduction

The vital healthcare requires of more than 80% of universal population depend primarily on plant medicine as estimated by the World Health Organization (Utkarsh Kaushile,2013). Plants are one of the most important sources of medicine; the use of medicinal herbs has become an important part of the daily life despite the progress in modern medical and pharmaceuticals research. *Acacia concinna* (Willd.) DC. is a medicinal plant that grows in tropical rainforests of Southern Asia and the fruits of this plant are used for washing hair, for promoting hair growth, as an expectorant, emetic and purgative (Gupta, G.L. et.al, 1971). The pods of *Acacia concinna* contain several saponins, highly polar compounds such as prosapogenol and monoterpene glycoside in various parts of the plants (Kiuchi, F,et.al, 1997). Phytochemical screening of pods yielded alkaloids, flavonoids phytosterols, saponin, tannins, phenolic compounds, gums and mucilage (Todar, et.al, 2010). In commercial extracts, when the plant is hydrolyzed, it yields lupeol, spinasterol, acacic acid, lactone and natural sugars glucose, arabinose and rhamnose. It also contains hexacosanol, spinasterone, oxalic acid, tartaric acid, citric acid, succinic acid, ascorbic acid and the alkaloids calyctomine and nicotine. The aim and Objectives are to extract the sample by using ethanol and distilled water and to determine the chemical compositions of *Acacia concinna* (Willd.) DC. fruits by Liquid Chromatography- Mass spectrometry.

Materials and Methods

Dried *Acacia concinna* (Willd.) DC. fruits were purchased from North Okkalapa Township in 2018. The fruits were dried in shade for several days when completely dried, these were pulverized by grinding machine to get the powder and stored in an air tight container for the chemical study. The extraction of compounds was carried out at the Department of Oriental Herb Science, Chonbuk University, Iksan in Korea.

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Extraction of *Acacia concinna* (Willd.) DC. fruits

100 g of each powder sample was extracted by using different solvents including 99% ethanol and distilled water. This samples were soaked in ethanol for 1 month and distilled water was boiled in water bath (60° C) for 1 hour. These extracts were filtered through a sheet of filter paper and then the filtrates were re-filtered through a 0.45 μm nylon membrane filter. The collected filtrates were dried in different processes. The ethanolic and aqueous extract were concentrated using a rotary evaporator with water bath at (60° C) and at (80° - 90° C). The two extracts were dried by freeze drier at (-62° C). And then, chemical constituents of *Acacia concinna* (Willd.) DC. fruits were analyzed by LC-MS/MS.

LC_MS/MS analysis

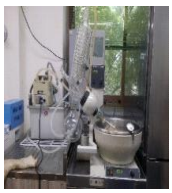
Unbiased metabolomics analysis was performed using an ultra-performance liquid chromatography (UPLC) system (Waters, Milford, USA). The chromatographic separation was carried out using ACQUITY UPLC HSS T3 column (100 mmx2.1mm,1.8μm, Waters) with a column temperature of 40°C and a flow rate of 0.5 ml/min, 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;100-35 reverse liner gradient phase B for 17~19 min; 97% phase A for 19- 25 min.

The loading volume of each sample was 5μl. The metabolites eluted from the column were detected by a high-resolution tandem mass spectrometer SYNAPT G 2 HDMS QTOF (Waters) in positive and negative ion modes. For positive ion mode, the capillary voltage and the cone voltage were set at 2 kV and 40 kV respectively. For negative ion mode, they were 1 kV and 40V, respectively. Centroid MS^E mode was used to collect the mass spectrometry data.

The primary scan ranged from 50 to 1200 Da and the scanning time was 0.2 s. All the parent ions were fragmented using 20-40 eV. The information of all fragments were 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. Data acquisition and analysis were controlled by Waters UNIFI V 1.71 software. The scan range in MS and MS/MS modes was over a range of 50-1200 m/z.



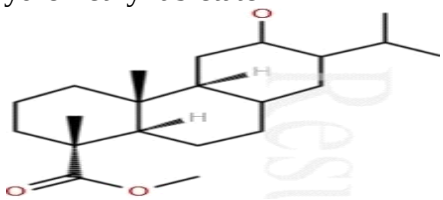
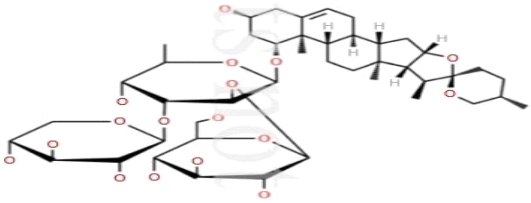
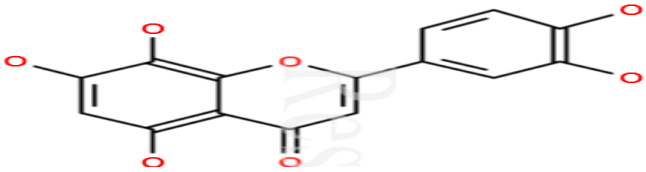
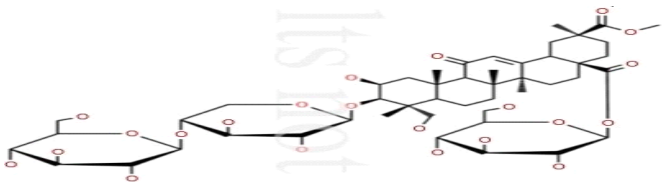
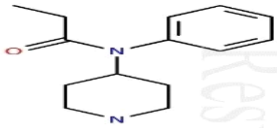
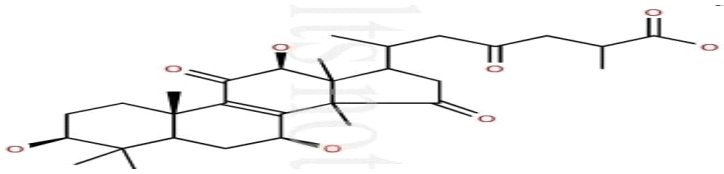



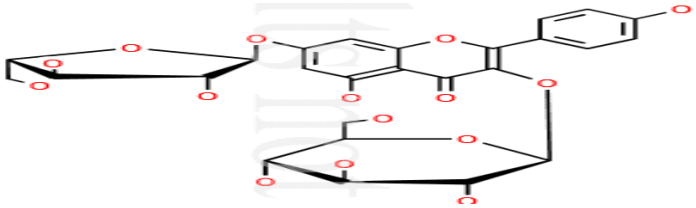
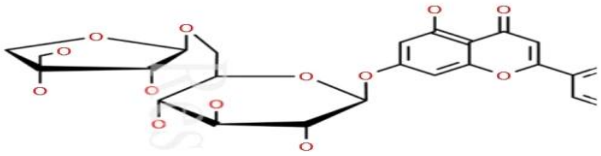
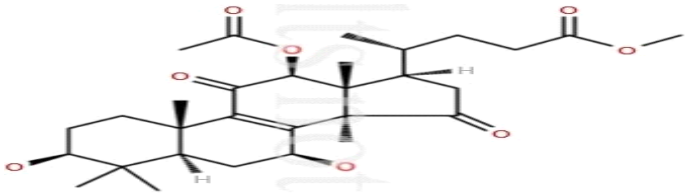
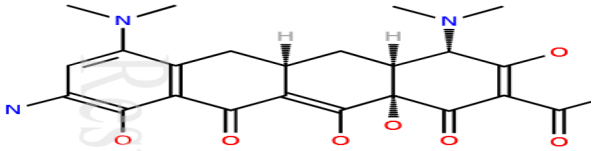
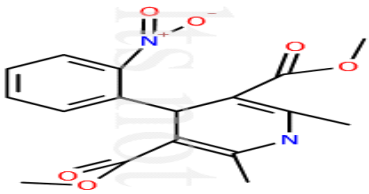
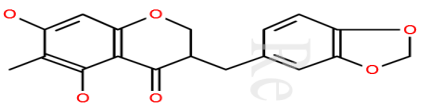
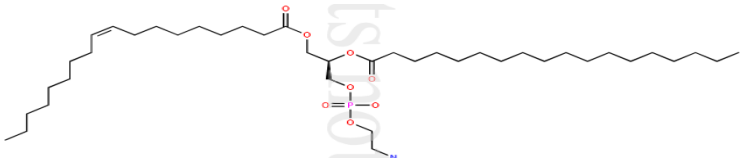

Results

LC-MS/MS analysis

According to LC-MS/MS analysis,19 possible compounds and 12 possible compounds were obtained from positive and negative ion modes from *Acacia concinna* (Willd.) DC. fruits. The results were shown in table (1) and (2).

Table (1). Item name: Acacia ES+, Sample position: 1: A, 4, Replicate number:1

	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
1.	12-Hydroxyhydromethyl abietate 	336.2664	336.2659
2.	12-Hydroxyhydromethyl abietate	336.2664	336.2662
3.	25(S)-Ruscogenin-1-O-[β-D-glucopyra-nosyl(1→2)][β-D-xylopyranosyl(1→3)]β-D-fucopyranoside 	870.4613	870.4584
4.	5,7,8, 3′,4′-Pentamethoxy flavone 	302.0427	302.0424
5.	Esculentoside M 	1002.4672	1002.4670
6.	Fentanyl, Nor 	232.1576	232.1570
7.	Ganoderic acid G 	532.3036	532.3034
8.	Ganoderic acid G	532.3036	532.3034
9.	Kaempferol-3-O-β-D-glucoside-7-O-α-L-arabinofuranoside	580.1428	580.1424

			
10.	Luteolin-7-O[β-D-apiofuranosyl(1→6)]β-D-glucopyranoside 	580.1428	580.1434
11.	Methyl lucidenate P 	532.3036	532.3034
12.	Minocycline, 9-amino 	472.1958	472.1952
13.	Nifedipine 	346.1165	346.1164
14.	Ophiopogonanone A 	328.0947	328.0940
15.	Phosphatidyl ethanolamines 	745.5622	745.5640
16.	Pterosin Y	280.1311	280.1305

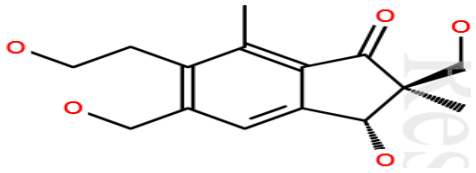
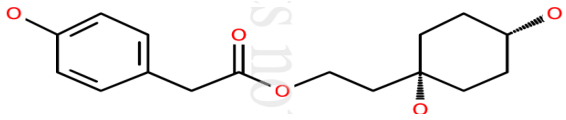
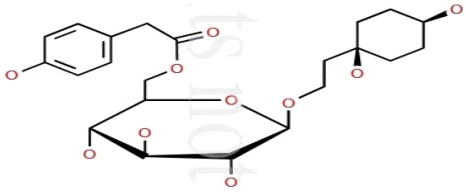
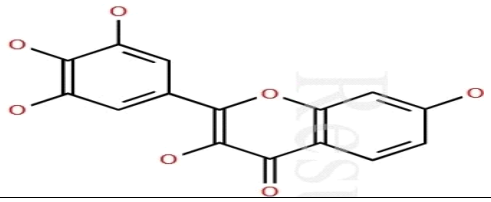
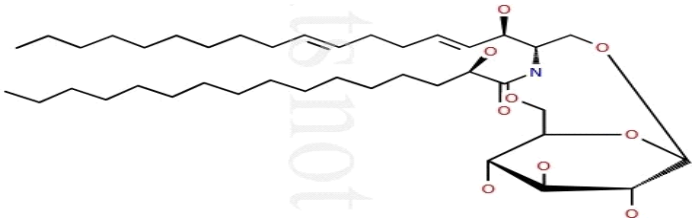
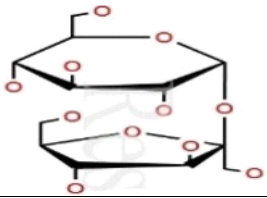
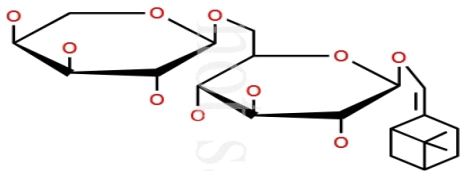
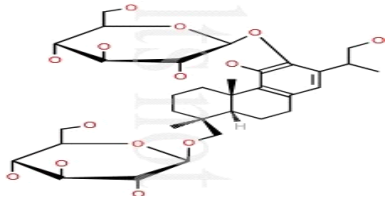
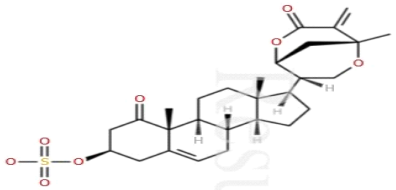
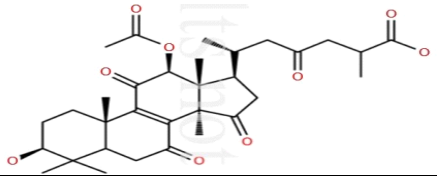
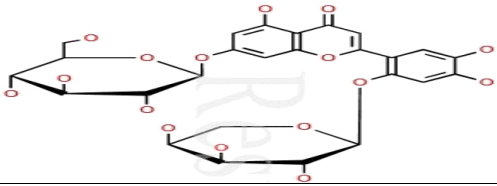
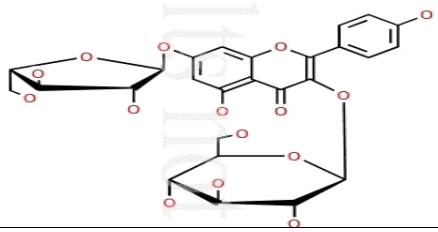
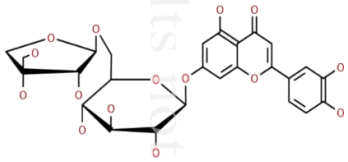
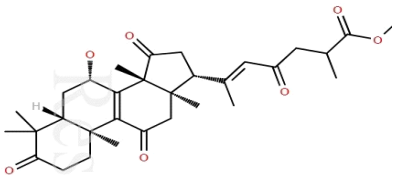
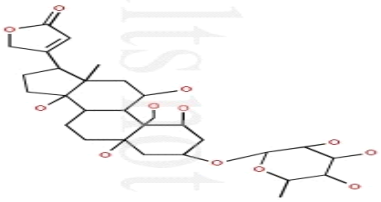
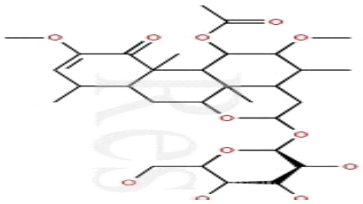
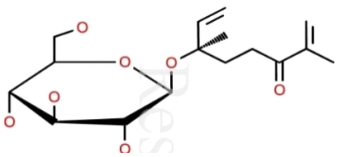
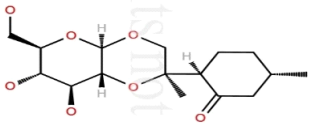
			
17.	Rengyolester 	294.1467	294.1463
18.	Rengyolester	294.1467	294.1462
19.	Rengyoside C 	456.1995	456.1999
20.	Robinetin 	302.0427	302.0424
21.	Soyacerebroside I- 1 	713.5442	713.5427
22.	Sucrose 	342.1162	342.1150

Table (2). Item name: Acacia ES-, Sample position: 1: A, 4, Replicate number:1

	Component name and Structure	Neutral mass (Da)	Observed neutral mass (Da)
1.	(Z)-(1S,5R)-β-Pinen-10-yl-β-vicianoside 	446.2152	446.2135
2.	(Z)-(1S,5R)-β-Pinen-10-yl-β-vicianoside	446.2152	446.2144
3.	Ajugaside A 	658.3201	658.3174
4.	Ajugaside A	658.3201	658.3180
5.	Ajugaside A	658.3201	658.3176
6.	Daturametelin F 	534.2287	534.2301
7.	Ganoderic acid H 	572.2985	572. 2961
8.	Isoetin-7-O-β-D-glucopyranosyl-2′-O-α-L-arabinopyranoside 	596.1377	596.1381
9.	Kaempferol-3-O-β-D-glucoside-7-O-α-L-arabinofuranoside 	580.1428	580.1428
10.	Kaempferol-3-O-β-D-glucoside-7-O-α-L-arabinofuranoside	580.1428	580.1432

11.	Luteolin-7-O[β-D-apiofuranosyl(1→6)]β-D-glucopyranoside 	580.1428	580.1428
12.	Methyl ganoderenate D 	526.2931	526.2906
13.	Ouabain 	584.2833	584.2816
14.	Picrasinoside D 	598.2989	598.2980
15.	Picrasinoside D	598.2989	598.2970
16.	Picrasinoside D	598.2989	598.2974
17.	Picrasinoside D	598.2989	598.2971
18.	Portuloside A 	330.1679	330.1672
19.	Schizonepetoside B 	330.1679	330.1673
20.	Schizonepetoside B	330.1679	330.1674

Discussion and Conclusion

In the present study, the analysis of chemical constituents from *Acacia concinna* (Willd.) DC. fruits by LC-MS/MS were described. In the previous study, the main constituents of *Acacia concinna* (Willd.) DC. fruits were found to alkaloids, glycoside, reducing sugar, α-amino acids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids and starch. According to LC-MS/MS analysis, 19 possible compounds and 12 possible compounds were obtained from positive and negative ion modes. In this results, *A. concinna* plant data and LC_MS/MS data were different. So, these compounds were not definitely identified by LC- MS/MS but these compounds may be checked by NMR.

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Todar, SS; et.al, 2010. Research Journal of Microbiology, vol. 5, No. 10. Pp. 974-979

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