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Screening of the *Cassia Fistula* Phytochemical Constituents by UPLC-ESI-QTOF-MS^2

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Abstract

Cassia Fistula leaves and their extracts are one of the most widely used herbal products and/or dietary supplements in the world. A systematic study of the phytochemical compounds is necessary to establish quality parameters. A UPLC-ESI-QTOF-MS^2 method was used to obtain chromatographic profiles for the compounds present in C. Fistula leaves. The method was used to identify 12 glycosylated phenolic compounds including one lignan, two phenolic acids and nine flavonoids in an aqueous leaf extract obtained under two extraction methods.

Keywords: *Cassia Fistula*; Phytochemical compounds; UPLC-ESI-QTOF-MS^2

Introduction

Many of the plants contain a variety of Phytopharmaceuticals, which have found very important applications in the fields of agriculture, health, food and nutrition development [1]. *Cassia Fistula L.* (*Fabaceae*, *Caesalpinioideae*), a very common plant known for its medicinal properties (antipyretic, analgesic, anti-inflammatory, and hypoglycemic effects) is native to India, the Amazon and Sri Lanka and diffused in various countries including Mexico, China, Mauritius, East Africa, South Africa and West Indies [2,3]. Over the past few years, there has been an exponential growth in study of primary and secondary metabolite composition that may be responsible for majority of the ascribed biological effects of this plant [4]. However, there are not enough studies that provide sufficient knowledge to the elucidation of specific phytochemicals present in this plant material. Hence, the present study was aimed to detect bioactive compounds present on extracts of *Cassia Fistula* leaves obtained by different extraction methods.

Materials and Methods

Chemicals: Acetonitrile, methanol, water, and formic acid were all LC-MS grade and purchased from Fisher Scientific Chemicals (Fair Lawn, NJ, USA).

Preparation of *Cassia Fistula* extracts: The powdered *Cassia Fistula* leaves were successively extracted by a conventional solid-liquid extraction (decoction), and the use of emerging technologies (microwave-assisted extraction) using a solid: liquid ratio of 1:50 w/v. The conditions used for both extraction methods are those reported in a previous study [5].

Phytochemical screening by UPLC-ESI-QTOF-M^2: Qualitative identification of phytochemicals was carried out using a BEH PHENYL (2.1 mm × 100 mm, 1.7 µm; WATERS, UK) analytical column operated at 40°C and the chromatographic separation was performed using a mobile phase of solvent A: 0.1% (v/v) formic acid water and solvent B: 100% acetonitrile, with a constant flow rate of 0.3 mL min with a gradient elution. While the full screen mass spectra detection (UHPLC system coupled to a quadrupole time-of-flight orthogonal accelerated QTOF mass spectrometer) was carried out in the negative ion mode in a mass range m/z of 50-1200 Da and using a capillary voltage of -3.5 kV and +4.0 kV. For more details, the complete conditions used are reported in a previous study [5].

Results and Discussion

The LC-MS analysis of phytocompounds in leaf extracts of *Cassia Fistula* explored the presence of various bioactive components. The identification of the phytocompounds was confirmed based on the molecular mass and its fragmentation pattern. The results (Table 1) show that *Cassia Fistula* contains 10 bioactive compounds which are extracted and differ according to the different extraction method applied in our study. The identified compounds include one lignan, two phenolic acids and seven flavonoids.

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Table 1: Phytochemical compounds detected and characterized in *Cassia Fistula* leaf extracts obtained by two extraction methods by using UPLC-Q/TOF-MS^2.

Peak N°	Rt (min)	[M-H]$^{-}$ $^{(m/z)}$	Tentative assignment	Polyphenol class	Molecular formula	MS^2 Dominant fragment ion	Occurrence	
							Decoction	MAE
1	1.89	417.007	Syringaresinol	Lignan	$C_{22}H_{26}O_8$	402.0354	x	x
2	2.37	463.027	Quercetin-O-hexoside	Flavonoid	$C_{21}H_{20}O_{12}$	300.9823		x
3	2.64	592.979	Apigenin-6,8-di-C- Glycoside	Flavonoid	$C_{27}H_{30}O_{15}$	472.9887	x	x
4	2.81	563.022	Kaempferol rhamnosyl xyloside	Flavonoid	$C_{26}H_{28}O_{14}$	430.1055	x	x
5	3.01	561.022	Coumaric acid derivative	Phenolic acid	--------	439.0179	x	x
6	3.15	562.98	Apigenin-6-C-pentoside-8-C-hexoside (Isomer 1)	Flavonoid	$C_{26}H_{28}O_{14}$	443.0515		x
7	3.15	562.981	Apigenin-6-C-pentoside-8-C-hexoside (Isomer 2)	Flavonoid	$C_{26}H_{28}O_{14}$	443.0203	x	
8	3.32	563	Apigenin-6-C-pentoside-8-C-hexoside(Isomer 3)	Flavonoid	$C_{26}H_{28}O_{14}$	443.019	x	x
9	3.42	562.981	Apigenin-C-hexoside-O-pentoside	Flavonoid	$C_{26}H_{28}O_{14}$	413.0619		x
10	3.42	478.982	Myricetin hexoside	Flavonoid	$C_{21}H_{20}O_{13}$	317.0013	x	
11	3.89	576.992	Proanthocyanidin B dimer	Flavonoid	$C_{30}H_{26}O_{12}$	425.0389	x	x
12	6.16	515.007	3,4-di-O-caffeoylquinic acid	Phenolic acid	$C_{25}H_{24}O_{12}$	353.0241		x

The LC-MS chromatogram of the 12 peaks of the compounds detected was shown in Figure 1. Chromatogram LC-MS analysis of the two extract of *Cassia Fistula* (Decoction and MAE) showed the presence of certain different peaks and the components corresponding to the peaks were determined as follows. Peak 1 ([M-H]- at m/z 417.0068) was identified as Syringaresinol. The characteristic fragment ions at m/z 402.0354 is resulting from the successive losses of two CH_3 groups. It is necessary to emphasize that seems to be the first report of the presence of Syringaresinol in the leaves of *Cassia Fistula*. Peak 2 with a precursor ion at m/z 463.0265 produced a fragmentation ion at m/z 300.9823 ([M-H]- m/z 162], suggesting the presence of Quercetin derivatives (Quercetin-O-hexoside). Apigenin-6, 8-di-C-glycoside was assigned to Peak 3. This compound exhibited a deprotonated molecule at m/z 592.9786 where its MS^2 spectrum produced a fragmentation ion at m/z 472.9887 which correspond for a fragmentation pattern of flavones di-C-glycoside (Apigenin as aglycone and two hexose moieties) [6]. Peak 4 presented a [M-H]- at m/z 563.0218, yielding a dominant fragment at m/z 430.1055 (from the loss of the second glycosyl, a rhamnosyl) suggesting that it could be a Kaempferol rhamnosyl xyloside. Peak 5 showed a base peak at m/z 561.0221 and displayed a fragmentation pattern at m/z 439.0179 that could be attributed to a loss of glycoside residues and was identified as Coumaric acid derivative. Apigenin-6-C-pentoside-8-C-hexoside (Isomers) (Peaks 6-8) and Apigenin-C-hexoside-O-pentoside (Peak 9) presented a pseudo-molecular ion at m/z 562.98 and fragmentation patterns at m/z 443 and m/z 413, respectively, indicating the presence of a C-hexosyl unit typical of the flavone asymmetric di-C-glycosides. Myricetin hexoside was assigned to Peak 10 were the di-glycoside were determined based on the detection of deprotonated ion at [M-H]- at m/z 478.9819 with a major characteristic flavonol ion fragment at m/z 317.0013 [7]. Peak 11 had [M-H]- at m/z 576.9918 and was identified as type B dimer of Proanthocyanidin [(epi) catechin-(epi) catechin] by comparison of its fragmentation behavior with previous works ([M-H]- at m/z 425.0389) [8]. Finally, the presence of 3,4-di-O-caffeoylquinic acid (Peak 12) was confirmed by its fragmentation pattern m/z 353.0241 → 191, indicating the presence of a monocaffeoylquinic acid (loss of caffeic acid moiety) [9]. The difference on composition in both extracts (Decoction and MAE) can be attributed to microwave radiation that has the property of transferring energy causing the instantaneous superheating in vegetal sample promoting chemical transformations which result in the organic synthesis of more compounds [10].

Conclusion

This study provides evidence of the possible presence of several active compounds in the crude extracts from *Cassia Fistula* leaves and revealing the presence of a wide variety of flavonoids. Finally, the use of chromatography and mass spectrometry is particularly useful for the rapid characterization of unknown active compounds of an extract.

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