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Original Article

Studies on Positive and Negative ionization mode of ESI-LC-MS/MS for screening of Phytochemicals on *Cassia auriculata* (Aavaram Poo)

Paranthaman Ramakrishnan, Sureshkumar Kalakandan*, Muthukumaran Pakkirisamy

ABSTRACT

Background: *Cassia auriculata* (Avaram) is an important medicinal plant in India Improved awareness in medicinal flowers has led to an increased need for efficient extraction methods and screening of flavonoid derivatives. **Objective:** To standardization of extraction solvent system and Characterization of flavonoids through positive and negative electrospray ionization mode using LC-MS/MS from *Cassia auriculata extract*. **Materials and Methods:** The different solvents like Methanol, Water, Acetonitrile, Ethyl Acetate, Ethanol, Chloroform, Hexane, Acetone Diethyl ether used frot he identification of flavonoids (Gallic acid, Theanine, Theobromine, Theophylline, Caffeic acid, Caffeine, Ferulic acid, Theacrine, Catechin, Quercetin, EpiGallo Catachin, catechin gallate, Epicatachin gallate and Quercetin hexoside. **Results:** Based on the peak area percentage the extraction solvent was standardized. The percentage of relative & absolute intensity of screened flavonoids was observed using LC-MS in positive and negative electrospray ionization. The results show that the methanol extract has more percentage of peak area, relative intensity and absolute intensity. The MS results showed that the negative ionoization has more intensity values of flavonoids and the signal-to-noise ratio was high in negative ionization mode compare to positive mode. **Conclusion:** Based on the results the methanol is the suitable extraction solvetnt and negative ionization mode of ESI-LC-MS/MS was appropriate for the screening of flavonoids on *Cassia auriculata* flower extracts.

Key words: Eelectrospray ionization, *Caucalis platycarpos* L.; Methanol, Flavonoids; UHPLC-ESI-MS.

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INTRODUCTION

The medicinal plant extracts and phytochemical both with identified antimicrobial properties are of huge importance. A number of studies have been done worldwide to establish antimicrobial behavior from medicinal plants. *Cassia auriculata* commonly known as" Avaram" that belongs to the *Caesalpiniaceous* family[1] and it have great in medicinal properties. The growth of ESI-MS has major impact in phytochemicals and its application has extended to a broad range of flavonoids as well as polar organic,[2] inorganic[3] and metal-organic complexes.[4] The ESI efficiency scale of the different organic molecules with different polarities.[5,6] The best ESI response has been observed for the analytes with ionizable polar functional groups. In positive ion mode the capillary is the positive electrode (anode) and the sampling aperture plate is the negative electrode (cathode). The positive ions within the eluent solution are repelled from the inner walls of the sprayer needle and move electrophoretically into the body of the droplet formed at the capillary tip. This mode causes positive ions was sprayed droplet and is used where the analytes form cations in solution. In negative ion mode the reverse situation occurs. The capillary is the negative electrode (cathode) and the sampling aperture plate is the positive electrode (anode). This mode causes negative ions to predominate the sprayed droplet and is used where the analytes form anions in solution. The Flowers of the plant are used in preparation of tea, which is prescribed in diabetes. Compound syrup is prepared with the flowers, mocharas and Indian saparilla which are prescribed for nocturnal emissions. The seeds are used in diabetes, opthalamia and chylous urine.[7] In the present study an effort has been made to standardization of solvent system (Methanol, Water, Acetonitrile, Ethyl Acetate, Ethanol, Chloroform, Hexane, Acetone Diethyl ether) and Characterization of flavonoids (Gallic acid, Theanine, Theobromine, Theophylline, Caffeic acid, Caffeine, Ferulic acid, Theacrine, Catechin, Quercetin, EpiGallo Catachin, catechin gallate, Epicatachin gallate and Quercetin hexoside) through positive and negative electrospray ionization mode using LC-MS/MS.

MATERIALS AND METHODS

Sample preparation

The dried flower was extracted with different solvents such as Methanol, Water, Acetonitrile, Ethyl Acetate,

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Ethanol, Chloroform, Hexane, Acetone Diethyl ether using Soxhlet method[8] for 72 h. The final extract was evaporated to dryness on a rotary vaccum evaporator at 40°C. The crude extract was re dissolved with 10 mL methanol and it was purified by solid phase extraction colum.

LCMS analysis of flavonoids

Flavonoids identification was done as described for LC-MS/MS analysis.[9] LC Conditions : Column: Inertsil ODS3, 150 mm × 2.1 mm, 5 µm, Mobile phase: A = 1% formic acid, B = acetonitrile, Gradient: Start with 5% B, At 30 min 50% B, Flow rate: 0.2 mL/min, Column temperature: 40°C and Injection volume: 10 µL. **MS Conditions:** Source: ESI,Ion mode: Positive & Negative , Vcapvoltage: 4000 V, Nebulizer: 50 psig, Drying gas flow: 10 L/min, Drying gas temp: 350°C, Corona: 4 µA, Vaporizer temperature: 350°C, Scan range: 100–1200 amu, Step size: 0.1, Peak width: 0.15 min Time filter: On, Fragmentor: 200 V.

RESULTS AND DISCUSSION

Screening of Flavonoids by HPLC-MS/MS

The phytochemical fingerprint of *Cassia auriculata* flower extract was estimated using UHPLC-ESI-MS conditions. The experiments were carried out in order to identification of flavonoids based on the intensity values arrived from LC-MS/MS. The LC-MS Total Ion Chromatogram (TIC) and mass spectrum of positive and negative ionization mode is a shown (Figure 1 to 3) by summing up intensities of all flavonoids mass spectral peaks belonging to the full scan.The Positive inonization shows Gallic acid (6.58%),Theanine (24.69%), Theobromine (32.1%), Theophylline (12.76%), Caffeic acid (59.26%), Caffeine (13.99%), Ferulic acid (49.38 %), Theacrine (43.25%), Catechin (90%), Quercetin (91.36 %), EpiGallo Catachin (37.45%), catechin gallate (79.01%), Epicatachin gallate (42.39%) and Quercetin hexoside (6.58%). The use of UHPLC and minimum sample research accounted for the possibility of this new accurate and fast process for the screening of flavonoids in the extract.

The methanol solvent was most effective in extracting phenolic components from plants. Methanol and ethanol have been proven as effective solvents for extraction of phenolic compounds.[10] The profile and yield of polyphenol content and antioxidant activity appears higher in more polar solvents.[11] The Figure 4 to 6 shows that Gallic acid (98.83%),Theanine (51.99%), Theobromine (97.15%), Theophylline (77.93%), Caffeic acid (90.32%), Caffeine (72.98%), Ferulic acid (39.85%), Theacrine (95.92%), Catechin (93.93%), Quercetin (90.36%), EpiGallo (Catachin83.75%), catechin gallate (84.08%), Epicatachin gallate (95.22%) and Quercetin hexoside (82.14%) the highest peak area percentage was found in methanol extract on negative ionization and followed by the acetonitrile, ethanol and other solvents. As a result, compared with the extraction yields of phytochemicals by extract solvent for 14 phenolic compounds, it was found that screening of phytochemicals, methanolic extraction for most favorable solvent. The analysis of phenolic acids and their esters were identified and characterized by negative ionization mode.[12,13,14] The

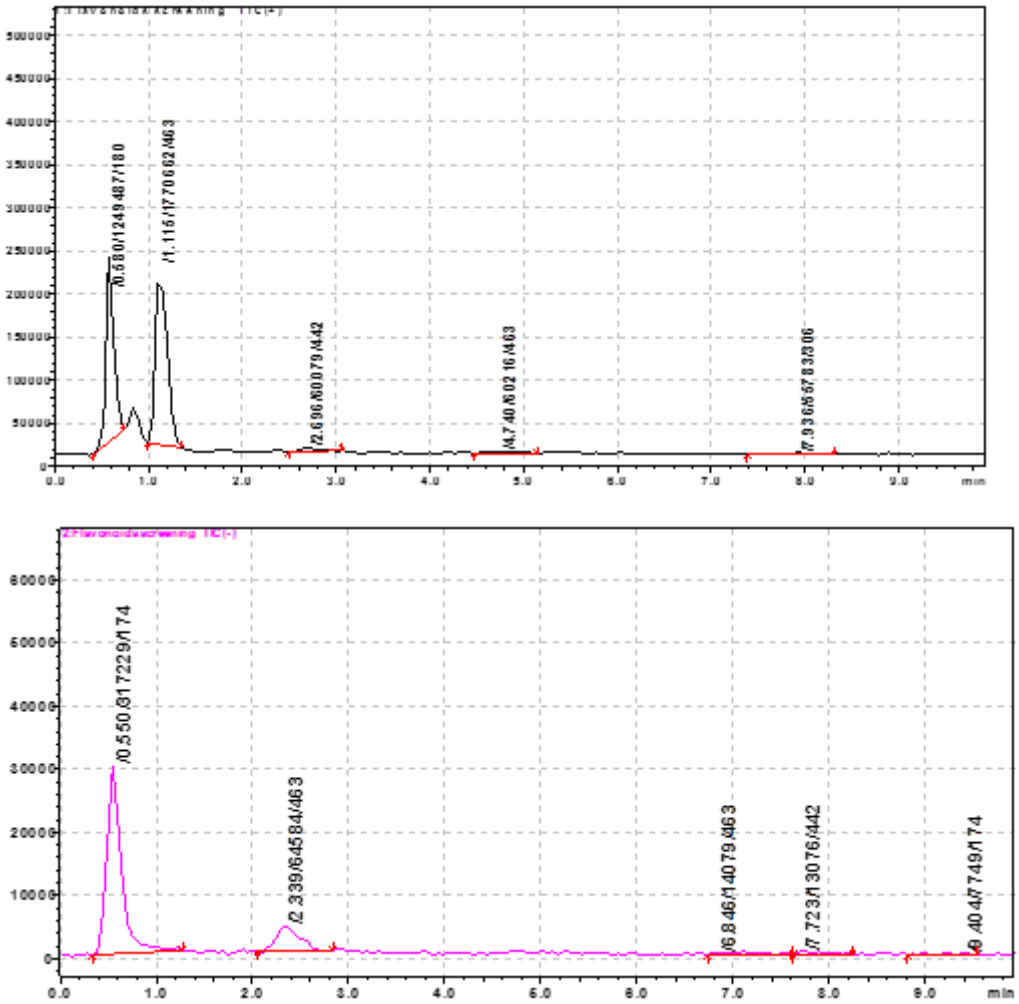

Figure 1: LC-MS Total Ion Chromatogram of flavonoids on Positive & negative ionization mode

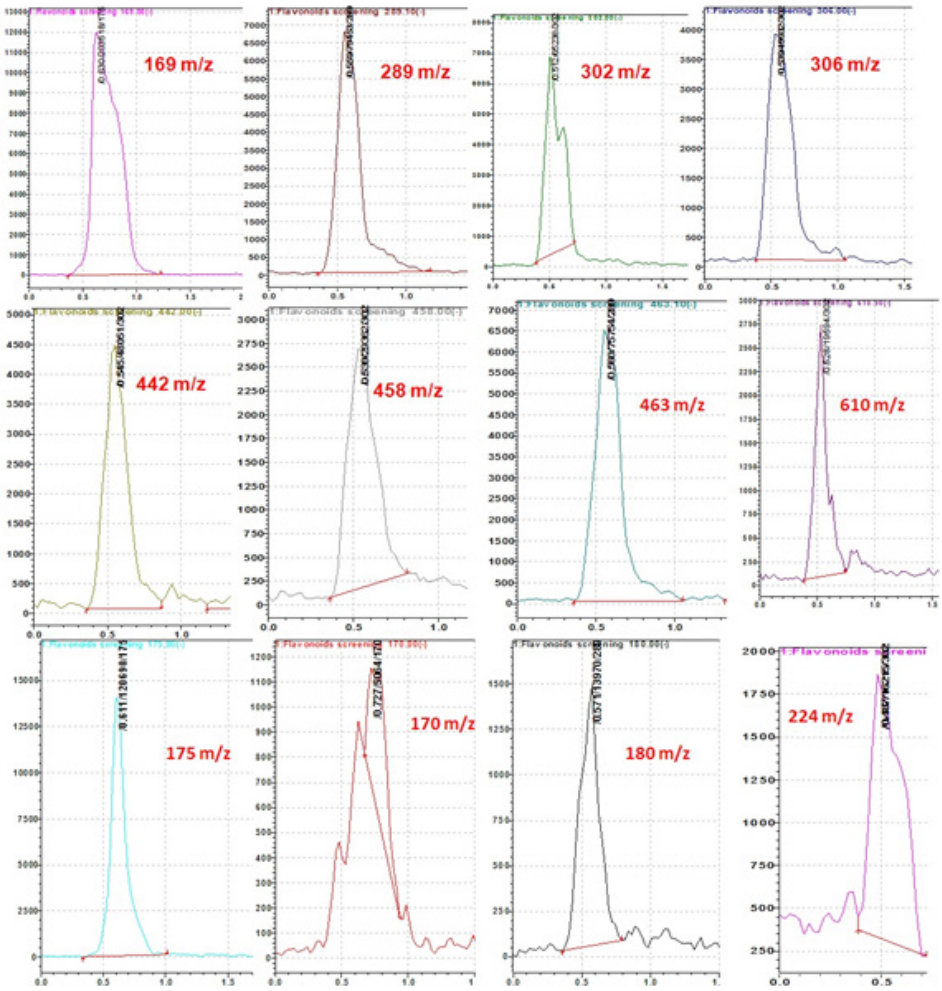

Figure 2: LC-MS Chromatogram of flavonoids on Negative ionization

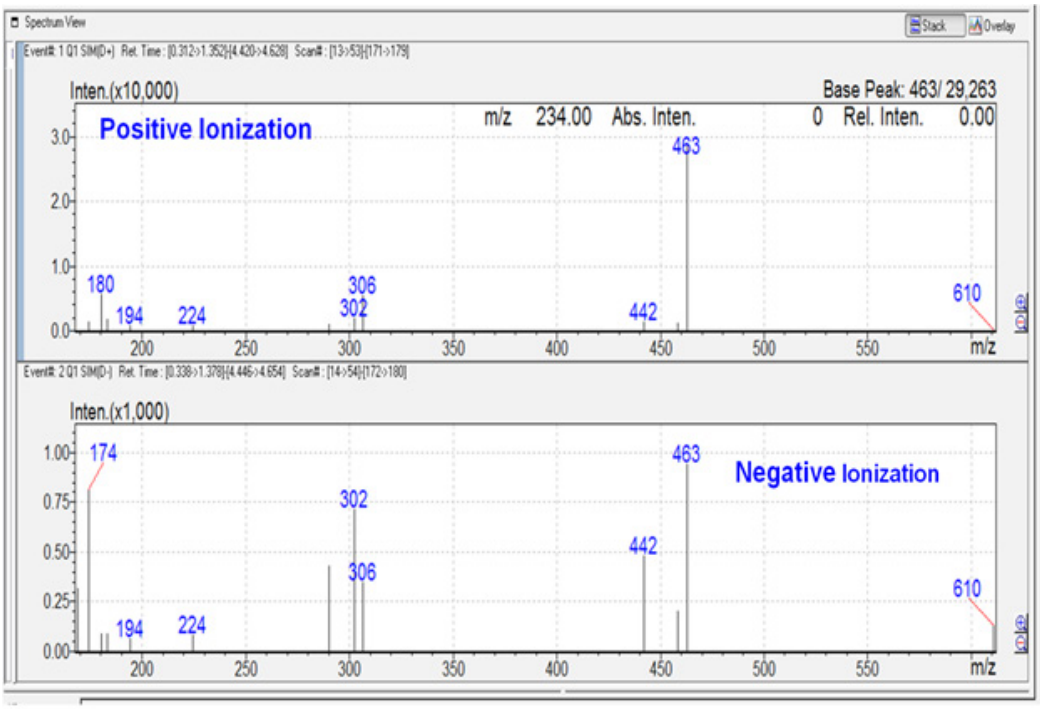

Figure 3: LC-MS mass spectrum of flavonoids on Positive & negative ionization mode

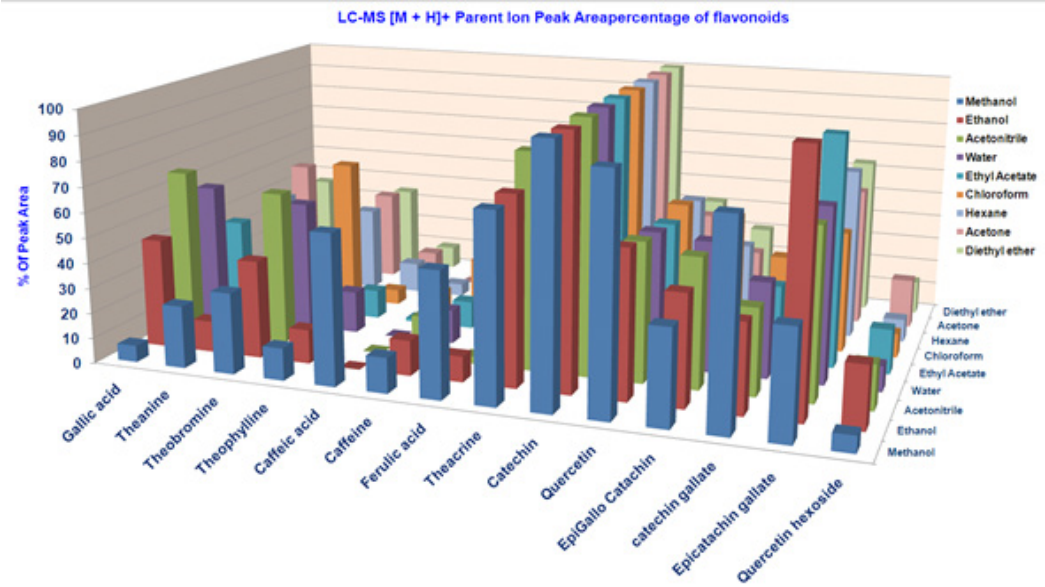

Figure 4: LC-MS [M + H]+ (Positive Ionization) Parent Ion Peak area percentage of flavonoids

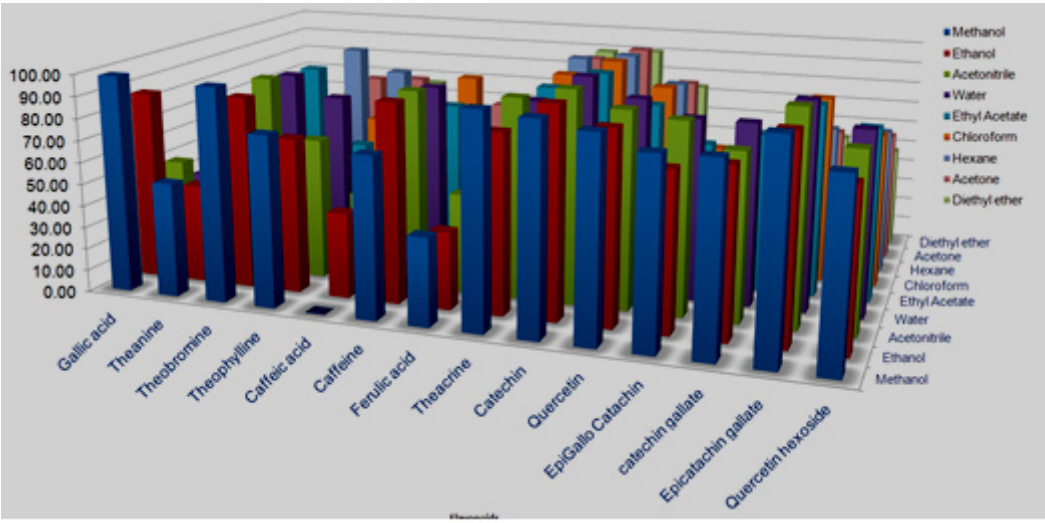

Figure 5: LC-MS [M - H]- (Negative Ionization) Parent Ion Peak area percentage of flavonoids

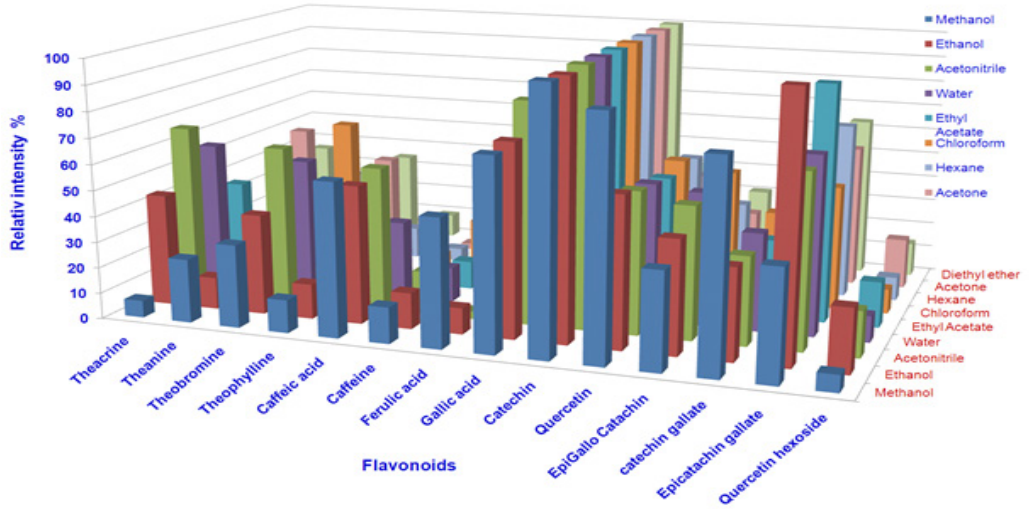

Figure 6: LC-MS [M - H]- (Negative Ionization) Parent Ion Relative intensity of flavonoids

Table 1: LC-MS Parameters for Flavonoids screening

Analyte	*m/z*	Cone Voltage (V)	Collision energy (eV)
Gallic acid	169	15	-35
Theanine	170	15	-35
Theobromine	175	15	-35
Theophylline	180	15	-35
Caffeic acid	183.5	15	-35
Caffeine	194	15	-35
Ferulic acid	224	15	-35
Theacrine	289.1	15	-35
Catechin	302	15	-35
Quercetin	306	15	-35
EpiGallo Catachin	442	15	-35
catechin gallate	458	15	-35
Epicatachin gallate	463.1	15	-35
Quercetin hexoside	610.5	15	-35

Table 2: LC-MS data of flavonoids on Positive & negative ionization mode

m/z	Retention Time (Rt)	Absolute Intensity		Relative Intensity		Compound
		Positive	Negative	Positive	Negative	
169	0.63	604	227	0.78	26.49	Gallic acid
170	0.727	2014	1545	5.28	88.33	Theanine
175	0.611	194	5867	20.05	8.51	Theobromine
180	0.571	224	2025	6.92	9.82	Theophylline
183.5	0.561	174	910	3.11	7.63	Caffeic acid
194	0.534	148	941	3.22	6.49	Caffeine
224	0.487	221	1065	3.64	9.69	Ferulic acid
289.1	0.559	1081	1150	3.93	47.41	Theacrine
302	0.512	1839	2165	7.4	80.66	Catechin
306	0.539	849	5619	19.2	37.24	Quercetin
442	0.545	1030	1616	5.52	45.18	EpiGallo Catachin
458	0.539	446	1322	4.52	19.56	catechin gallate
463.1	0.560	2280	29263	100	100	Epicatachin gallate
610.5	0.528	328	264	0.9	14.39	Quercetin hexoside

recovery of phenolic contents in different samples is influenced by the polarity of extracting solvents and the solubility of each compound in the solvent used for the extraction process.[15,16] High-performance liquid chromatography has been coupled with the ESI-MS for the molecular fractionation prior to mass-spectrometric analysis. Thus, HPLC/ESI-MS has efficient method competent of analyzing both small and large molecules of various polarities in a plant extract.

CONCLUSION

UHPLC-ESI-MS/MS in the Negative ionization mode enables the state screening of phytochemicals from Cassia auriculata flower extract using methanol. Liquid mass spectrometry served indeed a potent analytical instrument from qualitative and point of view for all types of phytochemicals investigated. By studying the methanolic Cassia auriculata flower extract, it was proved that the Eelectrospray ionization mass spectrometry (MS) based Qualitative analysis of phytochemicals is agreeable method in negative ionization of Single Ion Monitoring mode (Q1 SIM).

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CONFLICT OF INTEREST

We have a competing interest to declare and authors have no conflict of interest to declare.

ABBREVIATION USED

UHPLC: ULTRA High Performance Liquid Chromatography; **ESI:** Electron Spray Ionization; **MS:** MASS Spectrophotometer; **SIM:** Single Ion Monitoring mode; **Q1:** Quadrupole 1.

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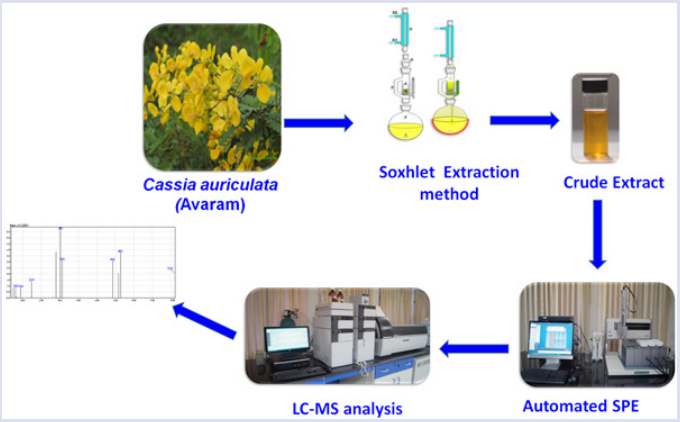
GRAPHICAL ABSTRACT



SUMMARY

- The present work has been performed to extensive qualitative study on the bioactive components of *Cassia auriculata* flower extract using UHPLC-ESI-MS/MS. The positive and negative electrospray ionization mode was optimized and negative ionization was suitable for the determination of flavonoids like Gallic acid,Theanine, Theobromine,Theophylline ,Caffeic acid , Caffeine , Ferulic acid , Theacrine , Catechin , Quercetin, EpiGallo Catachin, catechin gallate , Epicatachin gallate and Quercetin hexoside on methanol extract.

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