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Girme AS
Department of Pharmacology, Pravara Institute of Medical Sciences, A/P: Loni Dist: Ahmednagar, Maharashtra, India

Gaikar NV
Department of Quality Assurance, Pharmanza Herbal Pvt. Ltd., Anand, Gujarat, India

Saste GB
Department of Analytical R & D, Pharmanza Herbal Pvt. Ltd., Anand, Gujarat, India

Kunkulol RR
Department of Pharmacology, Pravara Institute of Medical Sciences, A/P: Loni Dist: Ahmednagar, Maharashtra, India

Correspondence
Girme AS
Department of Pharmacology, Pravara Institute of Medical Sciences, A/P: Loni Dist: Ahmednagar, Maharashtra, India

Chemical studies on antidiabetic botanical drug: *Cassia auriculata*

Girme AS, Gaikar NV, Saste GB and Kunkulol RR

Abstract
Cassia auriculata Linn (vernacular name: Avartaki,) is a traditional medicine used in the treatment of Diabetes mellitus. The present study is aimed to find out the phytochemical evaluation of *Cassia auriculata* Linn seeds using qualitative and quantitative tests on extracts. The chemical studies of different extracts were screened for the presence of phytochemicals. Fourier Transformer Infra-Red studies were done for functional group identification. The quantitative yield and its determination of various phytochemicals such as polyphenols were confirmed. Also a rapid and reverse phase Ultra High Performance Liquid Chromatography (RP-UHPLC) method developed with help of UV detector for six polyphenolic compounds namely gallic acid, caffeic acid, ferulic acid, ellagic acid, epicatechin and epigallocatechin.

Keywords: *Cassia auriculata*, phytochemical, chemical studies, polyphenols, rp-UHPLC

Introduction
Botanical drugs have been used in patients with Type-I and Type-II diabetes mellitus (DM) with other complications like diabetic retinopathy, diabetic peripheral neuropathy, etc. Compounds with different structure with different chemical moieties are responsible for the therapeutic activity in the treatment of DM. Its chemical studies can lead to further isolation and identification of potential lead towards its traditional claim [1]. Several phytoconstituents such as alkaloids, glycosides, flavonoids, saponins, dietary fibres, polysaccharides, glycolipids, peptidoglycans, amino acids and others obtained from various plant sources that have been reported as potent hypoglycaemic agents [2].
The validated botanical drugs are available now days in market with scientific studies and chemical standardisation. However, still potent traditional drugs like *Cassia auriculata* (CA) have been not studied for chemical studies for complete understanding of its phytochemistry.

Materials and methods
Collection and identification of plant material
Seeds were collected from District-Ahmednagar, Maharashtra. The plant material was authenticated at BSI-Pune by comparing morphological features (twigs arrangement, flower etc.). The herbarium of the plant specimen has been deposited at BSI-Pune. The dried seeds were cleaned, coarsely powder and used for chemical and phytochemical analysis.

Fig 1: Flowering plant and pods with different maturity of *Cassia auriculata*

Botanical Description

Scientific Name: *Cassia auriculata* Linn

Common Names: Tanner's Cassia (English), Tarvaṛ Taroda (Hindi), Taroda, Taravada (Marathi); Avaṛttakī, Vibhandi, Carmaranga (Sanskrit); Tangēdu (Kannada); Avarai (Tamil); Aval, Awal (Gujarati);Synonyms: Senna auriculata; Family: Caesalpiniaceae,

Description: The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, its flowers are irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The fruit is a short legume, 7.5–11 cm long, 1.5 cm broad, oblong, obtuse, pale brown. 12-20 seeds per fruit are carried each in its separate cavity.

Preparation of seed extracts

Seeds were collected from Dist. - Ahmednagar, Maharashtra. Seeds cleaned off adhering dust and unwanted plant material, shade dried, cut and pulverized (powdered). Further Seeds (500 g) were extracted with successive extraction at room temperature, filtered and concentrated under reduced pressure on rotary evaporator. The dried extract was successively fractionated in Petroleum Ether (40.5 gm) [CA-PE], n-butanol, (5.8g) [CA-NB] acetone: methanol 1:1 (26.8g) [CA-AM] and methanol: water 1:1 (21.23g) [CA-MW]. Also, separately seeds extracted with methanol by Soxhlet extraction at 60 ° C [CA-TS]. The solvents were chosen for larger delivery of bioactive compounds which are polar and mid-polar.

Phytochemical screening

The preliminary phytochemical analysis of petroleum ether extract [CA-PE], n-butanol extract[CA-NB], acetone: methanol extract[CA-AM], methanol: water extract [CA-MW] was screened for the presence of phytochemicals like Steroids, Triterpenoids, alkaloids, saponins, glycosides, sugars, flavonoid, tannins, Protein, Amino acid by standard methods [3].

1. Detection of steroids

Salkowski Test: Few mg of extract was taken in 2.0 ml of chloroform and 2.0ml of concentrated Sulphuric acid was added from the side of test tube. It was shaken for few min. The development of red colour in the chloroform layer indicated the presence of sterols.

Liebermann Burchard Test: Few mg of extract in a test tube, few ml of acetic anhydride was added and heated gently. Few drops of concentrated sulphuric acid were added to it appearance of blue colour gave evidence for presence of sterols.

2. Detection of Glycosides

Keller-Killani test- To 2 ml of extract, glacial acetic acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Appearance of reddish-brown colour at the junction of the two liquid layers indicates the presence of cardiac glycosides.

3. Detection of Alkaloids

Few mg of each extract was taken separately in dilute hydrochloride acid and filtered.

The acid layer was used for chemical tests for alkaloids

Dragendorff test: Acid layer with few drops of Dragendorff reagent gives reddish brown precipitate.

4. Detection of Triterpenoids

Liebermann-Burchard Test: Few mg of extract was dissolved in chloroform and few drops of acetic anhydride were added to it followed by concentrated sulphuric acid from side of test tube. Development of reddish-brown ring at the junction indicate the presence of terpenoids

5. Detection of Flavonoids

Shinoda Test: A small quantity of the extract was dissolved in 5ml of ethanol (95%v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings. Pink, magenta and crimson colour indicates presence of flavonoids.

6. Detection of Tannins

Ferric chloride Test: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet colour indicates the presence of Tannins Blue colour – Hydrolysable tannins, Brownish green- Condensed tannins

7. Detection of Proteins

Biuret test: To 2 ml of test solution (1% w/v), 2-5 drops of 1% w/v aqueous solution of copper sulphate and 2 ml of 40% w/v aqueous sodium hydroxide solution was added. The mixture was heated for 2 minutes. Formation of pink or violet colour indicated presence of proteins.

8. Detection of Amino acid

Ninhydrin test: Place 1 ml of each of the solutions in a test tube and add 2 drops of ninhydrin solution and boil the mixture over a water bath for 2 min. Allow to cool and observe the blue colour formed.

9. Detection of Saponins

Foam test: The extract was dissolved and mixed vigorously with water layer of foam or froth indicates the presence of saponins.

10. Detection of Sugars

Fehling's Test: To 2 ml of test solution, 2 ml of Fehling's solution A and Fehling's solution B was added and heated in a boiling water bath for 5 minutes. Formation of red precipitate indicated presence of reducing monosaccharides or disaccharides.

Fourier-transform infrared spectroscopy studies

The FTIR spectrum was used to identify and detect the characteristic peaks and functional groups of the active components based on the peak value in the region of infrared radiation.

The FTIR Spectra was performed using FTIR-ECO-ATR (Bruker, optic) connected to software of OPUS operating system. 1-1.5mg of test sample was positioned in contact with attenuated total reflectance (ATR) plate [4]. The spectra were recorded as absorbance values in duplicate.

Chemical studies

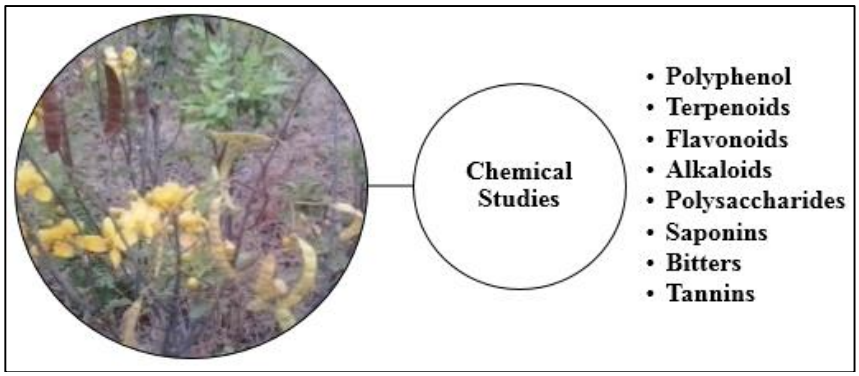

Fig 2: Chemical studies of Cassia articulate

The chemical studies were done to determine quantitative analyses of phytoconstituent found in preliminary phytochemical testing as shown in fig.2. All the results have been expressed as mean ± standard deviation (S.D).

Determination of Total polyphenols content

Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and based on structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans. The antidiabetic polyphenols play major role in diabetes because of their antioxidant nature [5]

Folin-Ciocalteu assay method was used for the determination of the total polyphenol content.100mg of the plant extract was used to determine total polyphenols content [6]. Set of three test tubes each containing 1ml of the standard solution, sample solution and water (as blank). 5ml Folin-Ciocalteu reagent was added into each tube and mixed well. 4.0mL of sodium carbonate solution into each tube was added after 8 minutes and mixed well. The reaction mixture could stand at room temperature in dark condition for 1 hour. The absorbance was measured at 765 nm against a blank solution. The content of total polyphenolics was extrapolated from the standard curve for Gallic acid and was expressed as mg Gallic acid equivalents (GAE) per gram of dry mass of extracts [7].

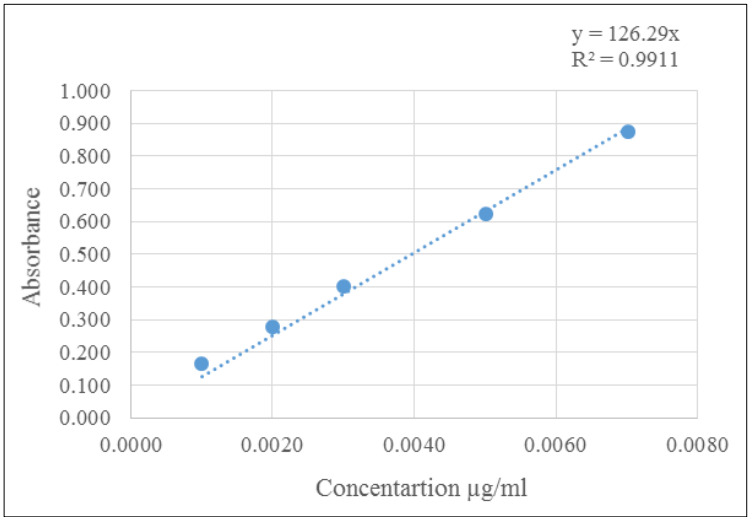

Fig 3: Linearity of Polyphenol method

Determination of total flavonoid content

Structurally, flavonoids consist of two aromatic rings (A and B rings) linked by a 3-carbon chain that forms an oxygenated heterocyclic ring (C ring). There are six subclasses of flavonoids including flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanidins based on differences in generic structure of the C ring, functional groups on the rings and the position at which the B ring is attached to the C ring. Within each subclass, individual compounds are characterized by specific hydroxylation and conjugation patterns. Flavonoids that can scavenge free radicals and chelate metals [8]. Quercetin, Diosmin, Fisetin, Quercetin-3-O-rutinoside, Luteolin and Naringenin [9]. Are some of well-known anti-diabetic flavonoids [10].The total flavonoids contents quantitatively estimated by UV Spectrophotometer method? 10mg of the sample was used to determine total flavonoids content. Test sample solution was prepared in test tube by adding 1ml of sample stock solution along with 3ml methanol, 0.2ml aluminium chloride, 0.2ml potassium acetate solution and 5.6ml distilled water and mix well in test tube. Sample blank was prepared in same way by replacing aluminium chloride with distilled water. Prepared solutions were filtered through Whatman filter paper and their absorbance was measured at 415nm against a blank solution. The content of total flavonoids contents was extrapolated from the standard curve for Quercetin [11].

Determination of total Alkaloids content

Alkaloids are organic compounds with nitrogen. The nitrogen atom will be at heterocyclic ring, with some exceptions. The alkaloids are mainly classified as true alkaloids and pseudo-alkaloids. The true alkaloids are derived from α-amino acid precursors. Other alkaloids, such as terpenes and steroids, are called pseudo-alkaloids because a relatively late amination process occurs in a transamination reaction by donating a nitrogen atom from an amino acid source. Alkaloids like vindoline I, vindolidine II, vindolicine III and vindolinine IV from catharanthis species [12]. Arecaidine [13]. Aegeline [14]. Berberine [15]. Have been shown their potential in DM. So, quantitative determination of total alkaloids was performed by gravimetric analysis.1gm of sample substance was poured into the conical flask. 100mL mixture of solvent ether: methanol [80:20] was added along with 5ml ammonia solution. Flask was frequently shake for one hour and solution was filtered. The residue was wash with mixture of ether: methanol [80:20] in 5 lots of 5mL each. 30mL of 1N sulphuric acid was added to total ether methanol solution in separating funnel. Acidic layer was collected and washed with of 20mL mixture of 0.5N sulphuric acid: methanol until complete extraction of the alkaloids is affected. Mixed acid solution was wash with 10mL chloroform. Acidic layer was made alkaline with dilute ammonia solution to the pH 7. Basified portion was partitioned with chloroform. All collected fractions were mixed together. Pooled chloroform fractions were evaporated to dryness [16]. Calculate the percent of alkaloids.

Determination of Saponin content

Saponins are naturally occurring surface-active glycosides. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature [15]. There have been several reports for saponin working as hypoglycemic agents [18, 19, 20, 21]. One gram of dry powder was reflux for half an hour with 50mL of 90% v/v methanol in flask. Residue was washed with 90% v/v methanol and all collected fractions were mixed together and evaporate on water bath to obtain a thick paste like residue. The methanol residue thus obtained was then fractionated using solvents of increasing polarity such as petroleum ether, Chloroform, Ethyl acetate and 90% v/v methanol. Precipitation was done using acetone and dried at 100^0c [22]. Calculate the percent of saponins

Determination of Total Polysaccharides estimation

The focus is also shifting towards polar or very polar compounds like polysaccharides for the development of effective therapeutics for DM [23, 24]. About 100mg of sample was dissolved in 100ml of distilled water. Take 3mL from the above and add 50µl of 80%w/v solution of phenol and heated on water bath at 60ºC for half an hour followed by 3ml of concentrated H_2SO_4drop wise after cooling. The absorbance was measured after 30 minutes at 480nm against blank [25].

Quantitative determination of total tannins by titrimetric analysis

It is well known antidiabetic effect present for condensed, hydrolysable tannins and tannins rich plants [26]. The estimation of these tannins was certain for knowing its concentration in CA [27]. 25mL of indigo sulphonic acid solution was added to above prepare sample solution and was mixed well. This mixture was titrated against 0.02M $KMnO_4$ solution till stable golden yellow colour develops. Deduct blank reading from the sample reading. One ml of 0.02M $KMnO_4$ is equivalent to 0.00415g of tannin substance. Calculate the percent of total tannin [28].

Quantitative determination of total bitters by gravimetric analysis

1 gm of the extract was refluxed with 50ml of Methanol on water bath for half an hour. It was filtered, and the above procedure was repeated twice. Methanol was then evaporated to obtain a thick paste approximate 5mL volume and dissolved in 25 mL of hot water completely. The extract was fractionated in a separating funnel with petroleum ether in separating funnel. Further petroleum ether layer was fractionated with Ethyl acetate thrice. All collected fractions were mixed together. Pooled ethyl acetate fractions were evaporated to dryness. Calculate the percent of total bitter [29].

Total Terpenoid content

Terpenes and terpenoids are the primary constituents of the essential oil compounds including terpenes, diterpenes and sesquiterpenes. They have unsaturated molecules composed of linked isoprene units, generally having the formula (C_5H_8) n. The compunds belong to the isoprenoid group. The triterpenoids [30]. And compounds like Limonene [31]. Mahanimbine [32]. Myrcene [33]. 5α-Cucurbitane [34]. from this class have been reported as anti-diabetic. So, the protocol developed for analysis of total terpenoids from CA as a Linalool [35].

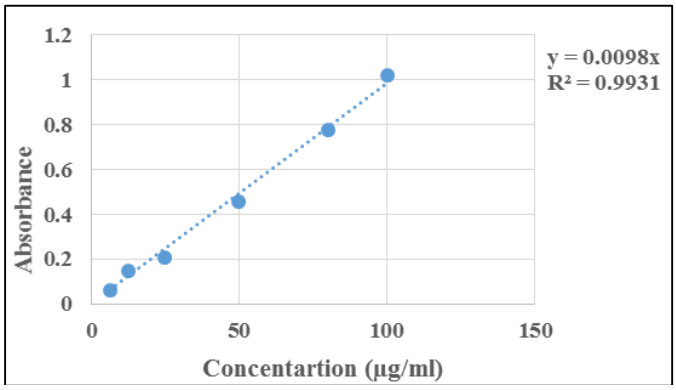

Fig 4: Linearity of Terpenoids method

Ultra High Performance Liquid chromatography (UHPLC) analysis

Chemicals &Reagents- Methanol and all other reagents of HPLC Grade were obtained from Rankem, India. All standards such as Gallic acid, Caffeic acid, Ferulic acid, Ellagic acid, Epicatechin and Epigallocatechin were procured from Sigma-Aldrich Company, USA. Double distilled water was used during analysis.

Instrument

The UHPLC system consists of Shimadzu corporation series with SIL-30AC auto sampler, LC-30AD quaternary solvent delivery unit, CTO-20AC column oven and SPD-20A UV detector with lab solution data software used. The analysis was performed on Reverse phase (RP) Phenomenex Luna-C18 column (250x4.6mm, 5µm).

Chromatographic conditions

The composition of the mobile phase was optimised by

varying the percentage of methanol, acetonitrile and water in reverse phase column. The optimization was done by different solvent system and operating system. Mobile phase Acetonitrile: 0.1% formic acid in water with gradient elution from 90% to 45% in 60 min, flow rate was 0.8ml/min, column temperature was 35^{0}c, Detector wavelength 272nm and 320nm.The GA, EA, EC and EGC were analysed at 272 nm and CF, FA analysed at 320 nm.

Sample Preparation

Test sample solution 10mg/ml was prepared in methanol.

Preparation of the Standard Solutions

Standard stock solution 0.1mg/ml of GA, CA, FA, EC and EGC were prepared separately in methanol and 0.1mg/ml of EA in double distilled water.

Results

Cassia auriculata (CA) seeds, family Caesalpiniaceae collected from Ahmednagar area, MH, India was authenticated by BSI-Pune (BSI/WRC/IDEN.CER/2016/53). The extractive yield for different extracts such as CA-PE, CA-NB, CA-AM, CA-MW, CA-TS was 9.2%, 8.15%, 11%, 9.65%, 13% respectively. Different chemical tests were performed on the extracts to find out nature of chemical constituents. The preliminary phytochemical investigation of different CA extract revealed the presence of sterols, triterpenes, flavonoids, tannins, glycosides and alkaloids as tabulated in Table1.

Table 1: Phytochemical results for various extracts

S.no	Phytochemical components	Chemical test	PE extract	NB extract	AM extract	MW extract
1	Steroids	Liebermann- Burchard Test	+	+	+	+
2	Glycosides	Keller-killani test	-	+	+	+
3	Alkaloids	Dragendroff's Test	_	_	+	+
4	Triterpenoids	Liebermann- Burchard Test	+	+	+	+
5	Flavanoids	Shinoda Test	+	+	+	+
6	Tannins	Ferric chloride Test	+	+	+	+
7	Proteins	Biuret test Millon's test	-	-	-	-
8	Amino acid	Ninhydrin test	-	-	-	-
9	Saponins	Foam Test	+	+	+	+
10	Sugars	Fehlings Test	-	+	+	+

(+ =Positive, - =Negative)

CA-PE: Petroleum ether extract, CA-NB: n-Butanol extract, CA-AM: Acetone Methanol extract, CA-MW: Methanol water extract

As per FTIR spectra (fig.5) of *Cassia auriculata* test sample extract, the functional groups of the components were separated based on their peak ratios. The result confirmed the presence of characteristic band at 3007.55 cm^{-1}, 2922.45 cm^{-1},2853 cm^{-1}shows the C-H stretching, at 2378.10 cm^{-1}, 2310.22 shows -P-H stretching, at 1742cm $^{-1}$ shows the C=O stretch carboxylic acid / Ketone group, at 3397.87cm^{-1} shows O-H stretch group, at 1457.46 cm^{-1} shows C-H bending, at 1055.21 cm^{-1}, 1032.84cm^{-1}, 1097cm^{-1}shows C-C stretch group.

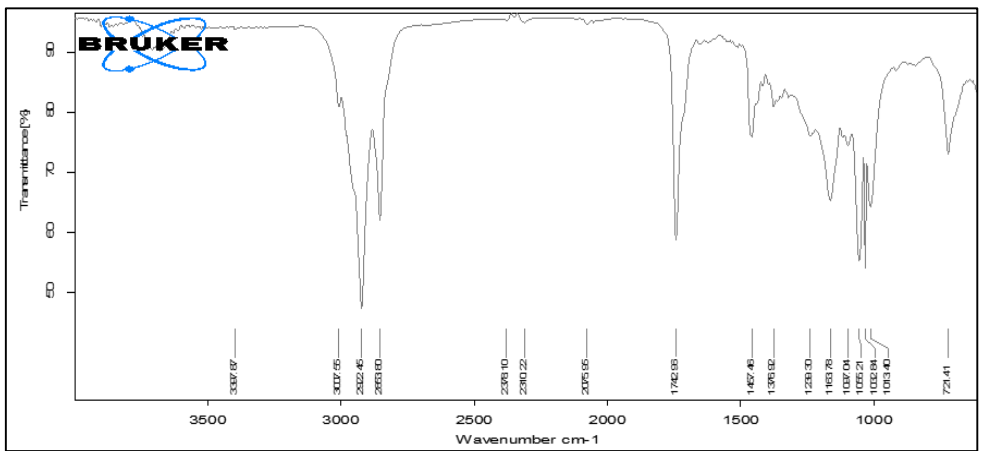

Fig 5: FTIR spectra of *Cassia auriculata* extract

Table 2: Results of chemical studies

S.no	Name of extracts	Terpenoids	Polyphenols	Flavonoids	Alkaloids	Tannins	Saponins	Polysaccharides	Bitters
1	CA-PE	17.54	14.87	10.51	--	2.31	2.35	--	--
2	CA-NB	24.66	21.85	12.65	--	3.14	10.25	12.25	--
3	CA-AM	18.31	28.04	13.51	5.45	5.62	6.54	25.21	1.89
4	CA-MW	19.59	34.41	12.78	6.12	10.15	8.42	21.24	2.14
5	CA-TS	31.59	38.23	15.27	6.23	15.12	11.41	24.15	2.51

Results are expressed as n=3 (%w/w) Mean ± S.D.

CA-PE: Petroleum ether extract, CA-NB: n-Butanol extract, CA-AM: Acetone Methanol extract, CA-MW: Methanol water extract CA-TS: Test sample

Results of chemicals studies as per Table 2 indicates that, CA-TS fraction showed higher content of terpenoids, polyphenols, flavonoids, alkaloids, tannins, saponins, bitters as compared to other extracts whereas CA-AM extracts shown maximum content of Polysaccharides.

As polyphenols were the major group present quantitatively in different extracts of CA, a rapid and Reverse phase Ultra High Performance Liquid Chromatography (RP-UHPLC) method was developed with help of UV detector. The chromatographic conditions for CA test sample and standard during analysis applied was same. The chromatogram of CA test sample scanned at 320nm and 270nm showed different peaks with retention time (fig.6 to 7).The identification of the peaks in chromatogram through eluted compounds were identified through comparison of retention time with standards (fig.8 to 10). The retention time of GA, EGC, EP, EA at 272nm were 5.7 min, 10.6 min, 18.1 min, 37.2 min and of CF, FA at 320nm were 17.9 min, 32.4 min respectively. Quantification of all markers in µg/mL from CA test sample as tabulated in Table 3 was performed.

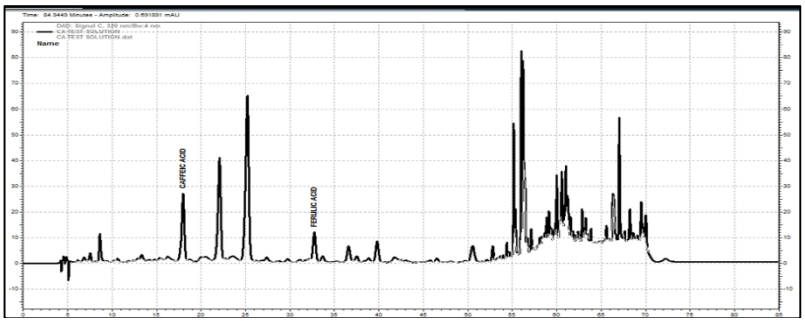

Fig 6: Test sample (TS) Chromatogram at 320 nm

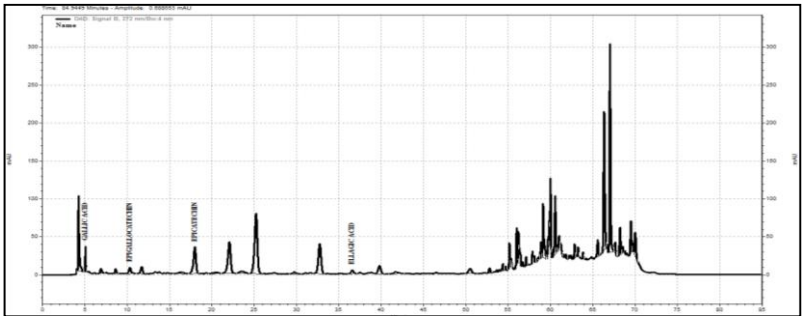

Fig 7: Test sample (TS) Chromatogram at 272 nm

Standard chromatograms

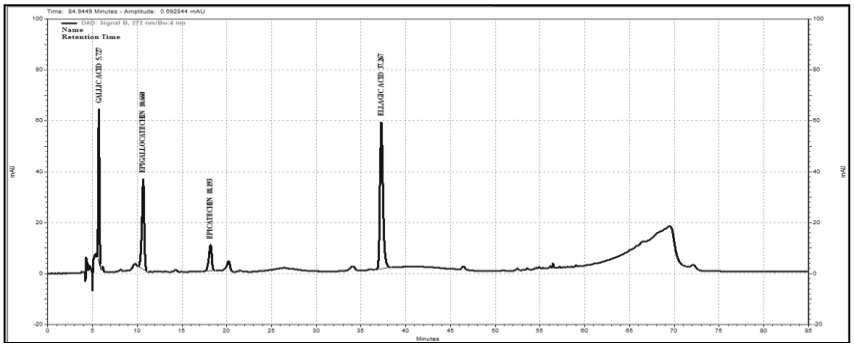

Fig 8: Standard Gallic acid (GA), Ellagic acid (EA), Epicatechin (EP), and Epigallocatechin (EGC) chromatogram at 272 nm

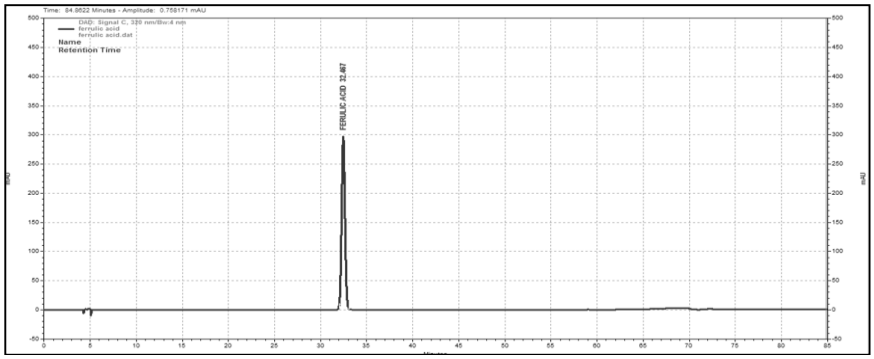

Fig 9: Standard Ferulic acid (FA) chromatogram at 320 nm

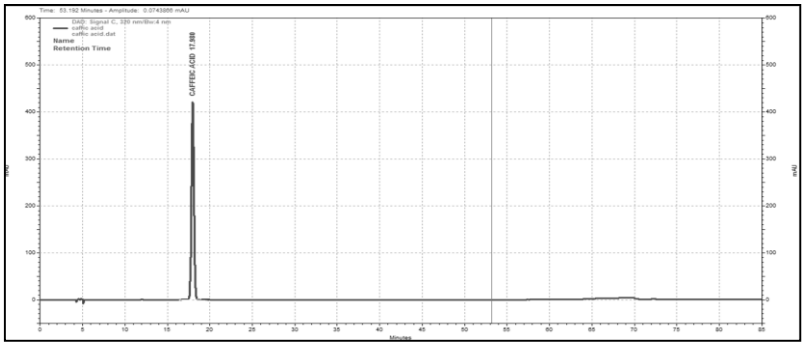

Fig 10: Standard Caffeic acid (CF) chromatogram at 320 nm

Table 3: Quantification of markers in (%w/w) from Test sample (TS)

S. No	Name	Gallic acid	Caffeic acid	Ferulic acid	Ellagic acid	Epicatechin	Epigallocatechin
1	CA-TS	0.8	0.05	0.02	0.005	0.01	0.02

CA-TS: Test sample

Discussion

The detailed chemical investigation involves the process from collection and authentication of plant material to its extraction by different solvent and its evaluation by qualitative and quantitative methods for phytochemical evaluation of crude drugs.

The present work represents the systematic study of plant material with qualitative and quantitative analysis of preliminary phytochemical investigation to fingerprinting of functional group with FTIR spectra, further laboratory experiment of quantitative determination of chemical groups supporting claims to sophisticated analytical techniques for the optimization, development and identification of phytoconstituent in *Cassia auriculata*.

Cassia auriculata (CA) is a traditional ayurvedic medicine used for DM. Phytochemical screening showed presence of different phytochemicals such as polyphenols, flavanoids, terpenoids, tannins, polysaccharides, saponins. Chemicals studies were carried out on different extracts for quantitatively determination of polyphenol, terpenoids, flavonoids, alkaloids, polysaccharides, saponins, bitters and tannins. Reverse phase UHPLC method was optimise and developed for the detection of polyphenols in different extracts of CA. The common phenolic markers such as CF, FA, GA, EGC, EP, EA chosen for standardisation of CA different extracts including test sample. The identification done through method development by retention time in comparison to standard chromatogram. The developed method was found well resolved for this quantification.

Conclusion

Chemical studies on the seeds of *Cassia auriculata* showed the presence of different phytochemicals mainly polyphenols. Similarly quantative analysis on chemical studies showed polyphenols content was maximum in CA test sample as 8.43%w/w. Further RP-UHPLC method was developed for quantification and identification of polyphenols markers such as CF, FA, GA, EGC, EP, EA in *Cassia auriculata* test sample.

List of Abbreviations

S. No	Abbreviation	Title
1	CA	*Cassia auriculata*
2	DM	Diabetes mellitus
3	RP-UHPLC	Reverse phase Ultra High Performance Liquid Chromatography
4	CA-PE	Petroleum Ether extract
5	CA-NB	n-Butanol extract
6	CA-AM	Acetone Methanol extract
7	CA-MW	Methanol water extract
8	CA-TS	Test sample
9	FTIR	Fourier-transform infrared spectroscopy
10	GA	Gallic acid
11	CF	Caffeic acid
12	FA	Ferulic acid
13	EA	Ellagic acid
14	EC	Epicatechin
15	EGC	Epigallocatechin

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References

1. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. Journal of Ethnopharmacology. 2006; 106(1):1-28.
2. Gaikwad SB, Krishna MG, Sandhya RM. Phytochemicals for diabetes management. Pharmaceutical Crops. 2014; 5(1):11-28.
3. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Nirali Publication, Pune, 2001, 149-156.
4. Cao Z, Wang Z, Shang Z, Zhao J. Classification and identification of Rhodobryum roseum Limpr. And its adulterants based on fourier-transform infrared spectroscopy (FTIR) and chemometrics. PLOS-ONE. 2017, 1-14

5. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009; 2(5):270-278.
6. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology. 1999; 299:152-178.
7. Blainski A, Lopes GC, De Mello JC. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules. 2013; 18(6):6852-65.
8. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies–. The American journal of clinical nutrition. 2005; 81(1):317S-25S.
9. Kawser HM, Dayem A, Han J, Yin Y, Kim K, Kumar SS *et al* Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. International journal of molecular sciences. 2016; 17(4):569.
10. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutrition & metabolism. 2015; 12(1):60.
11. Kalita Pallab, Barman Tapan K, Pal Tapas K, Kalita R, Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of biophytum sensitivum linn. Journal of Drug Delivery & Therapeutics. 2013; 3(4):33-37
12. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF, *et al.* Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. Molecules. 2013; 18(8):9770-84.
13. Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chemical research in toxicology. 2006; 19(6):818-27.
14. Narender T, Shweta S, Tiwari P, Reddy KP, Khaliq T, Prathipati P, *et al.* Antihyperglycemic and anti-dyslipidaemia agent from Aegle marmelos. Bioorganic & medicinal chemistry letters. 2007; 17(6):1808-11.
15. Cicero AF, Tartagni E. Antidiabetic properties of berberine: from cellular pharmacology to clinical effects. Hospital Practice. 2012; 40(2):56-63.
16. Rajpal V. Standardization of botanicals: testing and extraction methods of medicinal herbs. 2nd Edition, Volume 1, Business horizons, 2011, 256.
17. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. British journal of Nutrition. 2002; 88(6):587-605.
18. Lee KT, Sohn IC, Kim DH, Choi JW, Kwon SH, Park HJ. Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their antioxidant activity *in vitro* Archives of Pharmacal Research. 2000; 23(5):461-6.
19. Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H. Medicinal flowers. III. Marigold. (1): hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D from Egyptian Calendula officinalis Chemical and Pharmaceutical Bulletin. 2001; 49(7):863-70.
20. Bhavsar SK, Singh S, Giri S, Jain MR, Santani DD. Effect of saponins from Helicteres isora on lipid and glucose metabolism regulating genes expression Journal of ethnopharmacology. 2009; 124(3):426-33.
21. Elekofehinti OO. Saponins: Anti-diabetic principles from medicinal plants-A review. Pathophysiology 2015; 22(2):95-103.
22. Matsui Y, Kobayashi K, Masuda H, Kigoshi H, Akao M, Sakurai H, *et al.* Quantitative analysis of saponins in a tea-leaf extract and their antihypercholesterolemic activity. Bioscience, biotechnology, and biochemistry 2009; 73(7):1513-9.
23. Wang PC, Zhao S, Yang BY, Wang QH, Kuang HX. Anti-diabetic polysaccharides from natural sources: A review. Carbohydrate polymers. 2016; 148:86-97.
24. Wang J, Hu W, Li L, Huang X, Liu Y, Wang D, *et al.* Antidiabetic activities of polysaccharides separated from Inonotus obliquus via the modulation of oxidative stress in mice with streptozotocin-induced diabetes. PLOS ONE. 2017; 12(6):1-19
25. Corzo J, Leon-Barrios M, hernando-Rico V, Gutierrez-Navarro AM. Precipitation of metallic cations by the acidic Exopolysaccharides from *Bradyrhizobium japonieum* and *Bradyrhizobium* (*Chamaecyticus*) strain BGA-1**,** Applied and environmental microbiology. 1994; 60(12):4531-4536
26. Kunyanga CN, Imungi JK, Okoth M, Momanyi C, Biesalski HK, Vadivel V. Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. Journal of food science. 2011; 76(4):C560-7.
27. Ajebli M, Eddouks M. The promising role of plant tannins as bioactive antidiabetic agents. Current medicinal chemistry. 2018; 25:1
28. Rajpal V. Standardization of botanicals: testing and extraction methods of medicinal herbs.2nd Edition, Business horizons. 2011; 1:342-343
29. Rajpal V. Standardization of botanicals: testing and extraction methods of medicinal herbs.2nd Edition, Business horizons. 2011; 1:34
30. Goto T, Takahashi N, Hirai S, Kawada T. Various terpenoids derived from herbal and dietary plants function as PPAR modulators and regulate carbohydrate and lipid metabolism. PPAR research. 2010, 1-19
31. Panaskar SN, Joglekar MM, Taklikar SS, Haldavnekar VS, Arvindekar AU. Aegle marmelos C orrea leaf extract prevents secondary complications in streptozotocin-induced diabetic rats and demonstration of limonene as a potent antiglycating agent. Journal of Pharmacy and Pharmacology. 2013; 65(6):884-94.
32. Dineshkumar B, Mitra A, Mahadevappa M. Antidiabetic and hypolipidemic effects of mahanimbine (carbazole alkaloid) from Murraya koenigii (rutaceae) leaves. International Journal of Phytomedicine. 2010; 2(1):22-30
33. Boukhris M, Bouaziz M, Feki I, Jemai H, Feki AE, Sayadi S. Hypoglycemic and antioxidant effects of leaf essential oil of Pelargonium graveolens L'Hér. in alloxan induced diabetic rats. Lipids in Health and Disease 2012; 11(81):1-10
34. Ramírez-Espinosa JJ, Rios MY, López-Martínez S, López-Vallejo F, Medina-Franco JL, Paoli P, *et al.* Antidiabetic activity of some pentacyclic acid triterpenoids, role of PTP–1B: *In vitro*, in silico, and *in vivo* approaches. European journal of medicinal chemistry. 2011; 46(6):2243-51.
35. Ghorai N, Chakraborty S, Gucchait S, Saha SK, Biswas S. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Protocol Exchange. 2012; 5:1.