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Phytochemical Screening, HPTLC and GCMS Profile of *Acacia catechu (L.f)* Willd Hydroethanolic Leaf Extract

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A B S T R A C T

The plant *Acacia catechu (L.f) Willd* hydroethanolic leaf extract was subjected to qualitative and quantitative phytochemical screening, HPTLC and GCMS analysis. The phytochemical analysis revealed the presence of carbohydrates, proteins, flavonoids, total phenolics, glycosides, total free amino acids, tannins, alkaloids and thiols. The secondary metabolites like flavonoids, tannins and total phenolics were quantified as 82µg/ml, 67.5µg/ml and 42.5µg/ml. The HPTLC analysis of the leaf extract revealed the presence of flavonoids, tannins, phenolics and alkaloids. GCMS chromatogram showed sixty seven peaks which indicated the presence of thirteen phytochemical constituents. The major constituents were Furo[2,3-d] Pyrimidine-4,6 [5H,7H]-dion (10.20%), 2-Methyl-1,2,3,4-tetrahydro-beta-carboline(16.26%), Pthalic acid, butyl 2- pentyl ester (4.35%), Butylphosphonic acid and di(4-methoxy benzene) (14.82%). These results indicated that the *Acacia catechu (L.f) Willd* contain various bioactive components with wide range of medicinal properties, justifying the use of this plant to treat various diseases.

Introduction

Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantage over the synthetic drugs, that are much expensive and known to have harmful side effects (Akinmoladun *et al*., 2007). The biologically active phytochemical constituents include alkaloids, tannins, terpenoids, flavonoids and steroids that make specific physiological action on the human body.

Acacia catechu (L.f) Willd (family: *Leguminosae*) is widely distributed throughout Asia. Various pharmacological activities reported for the plant include immuno modulatory, hypoglycemic, antimycotic, antifungal, antiviral, antibacterial, anti-inflammatory and antioxidant activities (Singh *et al*., 1976; Ray *et al*., 2006; Wang *et al*., 2006). High Performance Thin Layer Chromatography (HPTLC) is an efficient quality assessment tool that allows the separation and detection of a broad number of phytochemical compounds (Malliga *et al*., 2015).

The HPTLC fingerprints could be used as an analytical tool for quality control and for determining the bioactive phytocomponents from the herbal medicine (Goodarzi *et al*., 2013). Gas Chromatography Mass Spectroscopy (GCMS) is the most commonly

used technique for the identification and quantification of unknown organic compounds in a complex mixture that can be determined by matching the spectra with reference spectra (Ronald Hites, 1997). The present study was sought to investigate the phytochemical and HPTLC profile as well as GCMS analysis of *Acacia catechu (L.f) Willd* hydroethanolic leaf extract.

Materials and Methods

Plant collection and preparation of extract

The plant *Acacia catechu (L.f) Willd* was collected from Kanjikode, Kerala, identified and certified by a taxonomist at Botanical Survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, (Plant identification No. BSI/SRC/5/23/2014-2015/Tech/699).

The leaves of *Acacia catechu (L.f) Willd* were shade dried and ground to a course powder by mechanical device. The extract was prepared using different solvents viz., peteroleum ether, chloroform, acetone, ethanol, 50% hydroethanol and water by cold maceration process. The filtrate was used for the preliminary phytochemical analysis (Khandelwal, 2005). Further studies were carried out using the 50% hydroethanolic extract, prepared using soxhlet apparatus. The extract was condensed to dryness using rotary evaporator and the crude residue obtained (15 % w/w) was stored in an air tight container until use.

Quantitative phytochemical screening

For establishing the phyto-constituents, the extract was subjected to quantitative phytochemical tests as per the standard procedure.

HPTLC analysis of *Acacia catechu (l.f)* Willd hydroethanolic leaf extract (Shah *et al*., 2008)

High performance thin layer chromatography is an automated form of TLC, used to purify the biologically active compounds qualitatively and quantitatively. It has better analytical precision and accuracy, where both sample and standard are processed simultaneously (Sutar *et al*., 2002).

Sample preparation and application

The plant extract 25 mg was dissolved in 250µl of 50% hydroethanol and centrifuged at 3000rpm for 5min. 0.1 µl of this solution and 2.0 µl of standard solution were loaded as 5mm band length in the 2 x 10 cm Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development and photo-documentation

The sample loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with the respective mobile phase (alkaloids, flavonoids, tannins and phenolics separately for each profile) and the plate was developed upto 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in a photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV 366nm / day light.

Derivatization and scanning

The developed plate was sprayed with respective spray reagent and dried at 100°C in hot air oven. The plate was photo-documented in day light/UV366nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC

SCANNER 3) and scanning was done at UV 366nm/day light. The peak chromatogram and peak densitogram were noted using the software WINCATS 1.3.4 version.

Gas chromatography mass spectral analysis (GCMS) (Vanitha *et al*., 2011)

GC-MS studies of medicinal plants are used for the analysis of non polar components, volatile essential oil, fatty acids, lipids (Jie and Choi, 1991) and alkaloids (Betz *et al*., 1997). GC-MS analysis of the plant extract was carried out using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0m, Diameter : 0.25 mm, Film thickness : 0.25µm composed of 100% Dimethyl poly siloxane).

For detection of the spectra, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2.0µl was employed. The injector and the ion source temperature were 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 minutes. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. The running time of the chromatogram was 35 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Software for mass spectra and chromatogram were GC MS solution ver. 2.53. The spectrum of the unknown component was compared with the spectrum of the known components, stored in the WILEY8 library. The name, molecular weight and molecular formula of the test material were identified.

Result and Discussion

The qualitative phytochemical analysis of *Acacia catechu* (L.f) *Willd* leaf extract revealed the presence of carbohydrates, proteins, thiols, tannins, total phenolics, alkaloids, flavonoids and glycosides as shown in the table -1.

In the quantitative analysis, the primary metabolites like carbohydrates, protein and total free amino acids were found to be 756µg/ml, 396µg/ml and 198µg/ml respectively as represented in the figure-1. Carbohydrates are biological macromolecules that not only serve as a source of energy but also possess antioxidant activity by which protects the cells against reactive oxygen species, chronic and degenerative diseases (Bin Li *et al*., 2012). Proteins are primary components of living organisms and are essential to maintain the structural and functional aspects of life including the growth and development (Bhumi and Savithramma, 2014). The amino acids are involved in the synthesis of proteins, amines, alkaloids, vitamins, enzymes and terpenoids (Ibrahim *et al*., 2010).

The secondary metabolites like flavonoids, tannins and total phenolics were quantified as 82µg/ml, 67.5µg/ml and 42.5µg/ml respectively as represented in figure-1. Flavonoids are powerful water soluble antioxidant, which helps in prevention of oxidative cell damage (Loots *et al*., 2007) through scavenging or chelating process (Kessler *et al*., 2003). Tannins are poly-phenolic compounds that are responsible for the prevention of chronic diseases (Vasundhara *et al*., 2013). The hydroxyl groups of phenolic compounds act as hydrogen donors, react with oxygen and nitrogen species, thereby break the cycle of generation of new free radicals (Pereira *et al*., 2009).

HPTLC fingerprinting profile for alkaloids

The HPTLC analysis of the plant extract for alkaloids (figure-2a) showed yellow and brownish yellow coloured zones at 366nm, which revealed the presence of 6 polyvalent phytoconstituents with the Rf values that ranged from 0.06 - 0.94. The component with Rf values of 0.06 and 0.35 were found to be more predominant with area spanning 131 and 533.3 respectively. The Rf value and peak area for standard colchicine were found to be 0.53 and 14929.3. The Rf value of 4th peak (0.35) coincide with the standard. The corresponding densitogram is presented in (figure-2b). Alkaloids have physiological and medicinal properties (Hounsome *et al*., 2008). Alkaloids possess antidiabetic and antibacterial properties (Akinyeye *et al*., 2014).

HPTLC fingerprinting profile for flavonoids

In flavonoids profile, yellow, yellowish blue colored zones were observed at 366 nm (figure-3a), which revealed the presence of 6 polyvalent phytoconstituents with the Rf values ranged from 0.15 - 0.96. The component with Rf values of 0.45 and 0.83 were found to be more predominant with area spanning 2570.8 and 2389.7 respectively.

Table.1 Qualitative phytochemical screening of *Acacia catechu* (L.f) Willd leaves extract

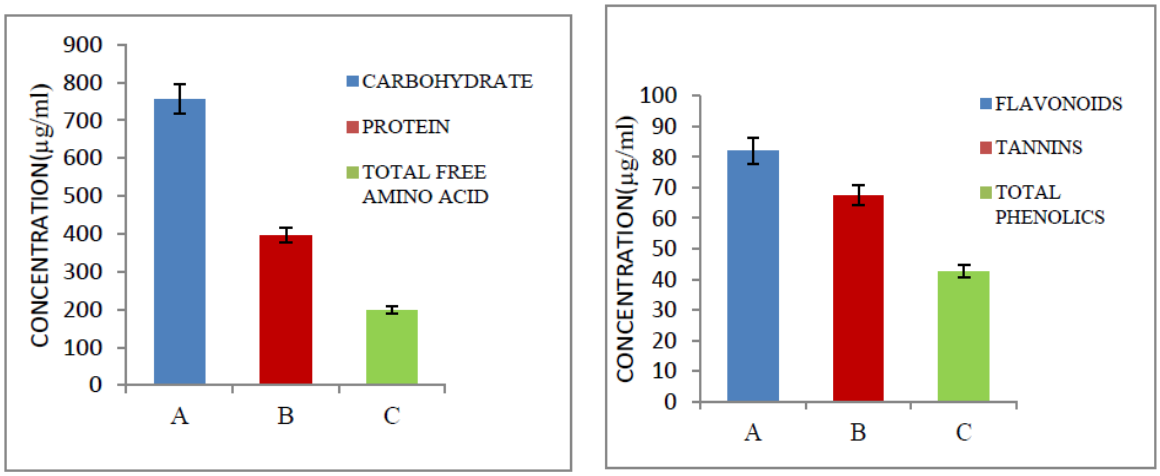
TESTS	WATER	ETHANOL	HYDRO ETHANOL	ACETONE	BENZENE	PET. ETHER
CARBOHYDRATE						
Fehlings Test	+	+	+	+	+	+
Benedicts Test	+	+	+	+	+	-
Molischs Test	+	+	+	+	+	-
PROTEIN						
Biuret Test	+	+	+	+	+	-
Ninhydrin Test	+	-	+	-	-	+
THIOLS						
Ellmans Test	+	+	++	++	+	-
ALKALOIDS						
Dragendroffs Test	-	-	+	++	+	-
Wagners Test	++	+	++	+	+	-
Meyers Test	+	-	++	+	+	+
PHENOLICS						
Ferric chloride Test	++	++	++	++	++	-
Lead Acetate Test	+	-	++	-	+	+
Libermanns Test	+	-	+	-	+	+
GLYCOSIDES						
Legals Test	++	++	++	++	++	-
Killer Killani Test	++	-	-	+	+	+
TANNINS						
Ferric Chloride Test	++	++	++	++	++	-
Lead Acetate Test	++	+	++	-	-	+
FLAVONOIDS						
Alkaline Reagent Test	+	+	++	++	+	+

(++) = highly present; (+) = present; **(–)** = absence of phytochemicals.

Table.2 Phytocomponents of *Acacia catechu* (L.f) Willd by GCMS analysis with the activity profile

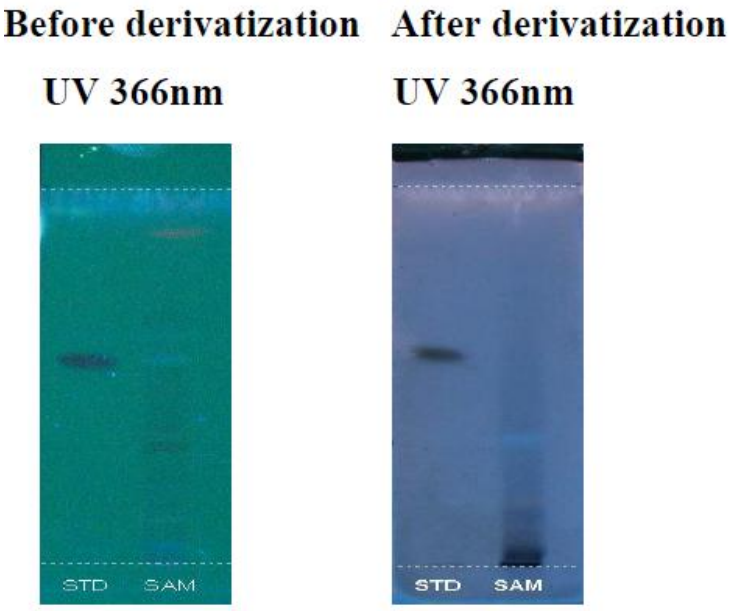
S. No	Ret. Time	Name of the compound	Area %	Activity
1.	13.275	Benzenamine, 3 ethoxy	1.13	Antibacterial and antifungal activity (Kaura and Kaura 2012).
2.	15.092	12-Methoxy-19-Norpo Docarpa	3.73	Anticancer activity (Antonio Salatino *et al*., 2007).
3.	25.083	Furo[2,3-d] Pyrimidine-4,6 [5H,7H]-dion	10.20	Antifungal and antibacterial activity (Sambavekar *et al*., 2014).
4.	26.158	2-Methyl-1,2,3,4-tetrahydro-beta-carboline	16.26	Antioxidant activity (Thomas Herraiz, 1999).
5.	26.242	2-Napthalenamine	3.36	Antioxidant activity (IARC, 2010).
6.	26.533	Phthalic acid, 6-ethyl-3-Octyl butyl ester	1.41	Antimicrobial activity (Gayathri Gunalan *et al*., 2014).
7.	26.650	1-Methoxy-4-(4-Methoxy benzene)	3.23	Antimicrobial activity (Panagal Mani *et al*., 2011).
8.	26.742	Pthalic acid, butyl 2- pentyl ester	4.35	Antimicrobial activity (Gayathri Gunalan *et al*., 2014).
9.	26.883	Hexadecanoic acid, ethyl ester	2.58	Antioxidant, Hypocholesterolemic (Dr. Duke's Phytochemical database).
10.	28.425	Phytol	1.62	Antioxidant activity, Antimicrobial, anticancer, anti-inflammatory effect (Amutha Aishwarya Devi and Kottai Muthu, 2014).
11.	29.125	9,12,15- Octadecatrienoic acid,	2.04	Anti-inflammatory effect (Rehana Banu and Nagarajan, 2013).
12.	29.600	Butylphosphonic acid, di(4-methoxy benzene)	14.82	Antioxidant activity (Rane Zab and Anusha bhaskar, 2012).
13.	29.642	2-Pyridine carbonitrile, 1,2,5,6	1.26	Antimicrobial activity (Borkhataria and Shah, 2014).

Fig.1 Quantification of primary and secondary metabolites present in hydroethanolic leaf extract of *Acacia catechu* (L.f) Willd



All values are mean ± standard deviation (n=3).

Fig.2a Chromatogram of *Acacia catechu* (L.f) Willd leaf extract for alkaloids



STD = Standard; SAM = Sample

Fig.2b Densitogram display of *Acacia catechu* (L.f)Willd leaf extract for alkaloids

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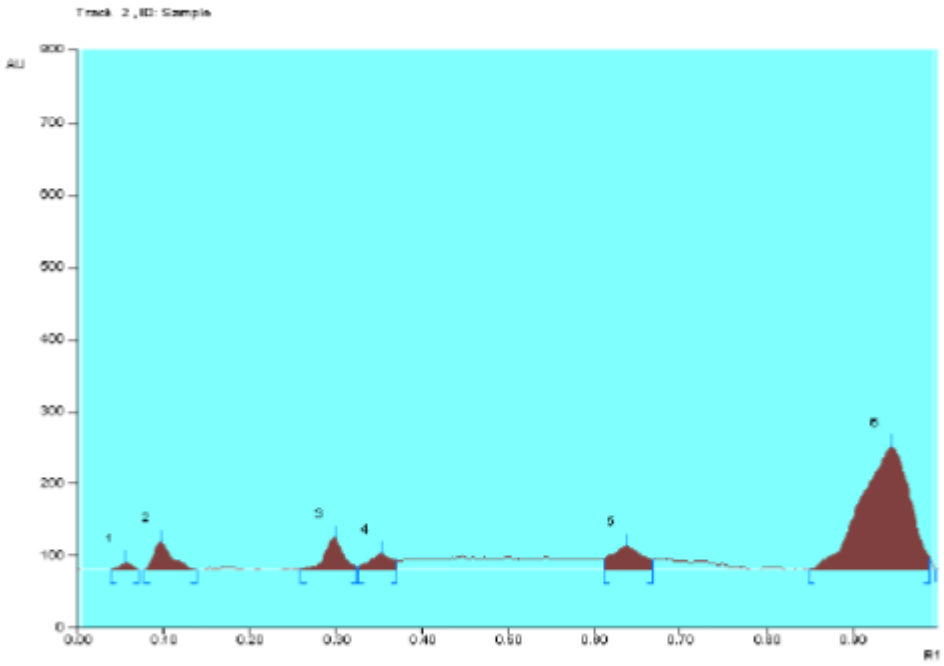
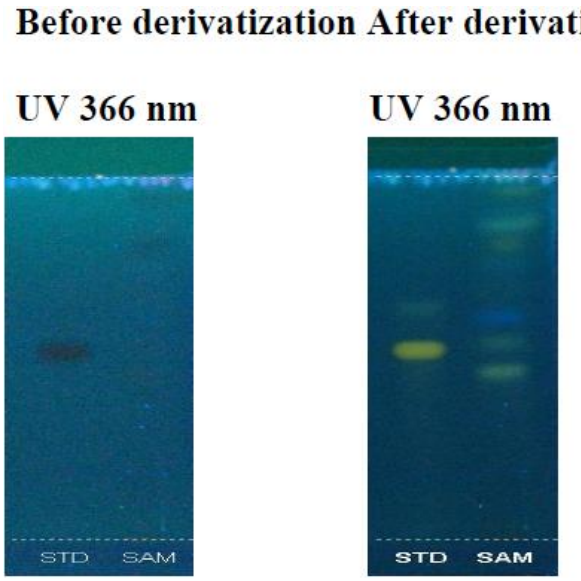


Fig.3a Chromatogram of *Acacia catechu* (L.f)Willd leaf extract for flavonoids.



STD = Standard; SAM = Sample

Fig.3b Densitogram display of *Acacia catechu*(L.f) Willd leaf extract for flavonoids

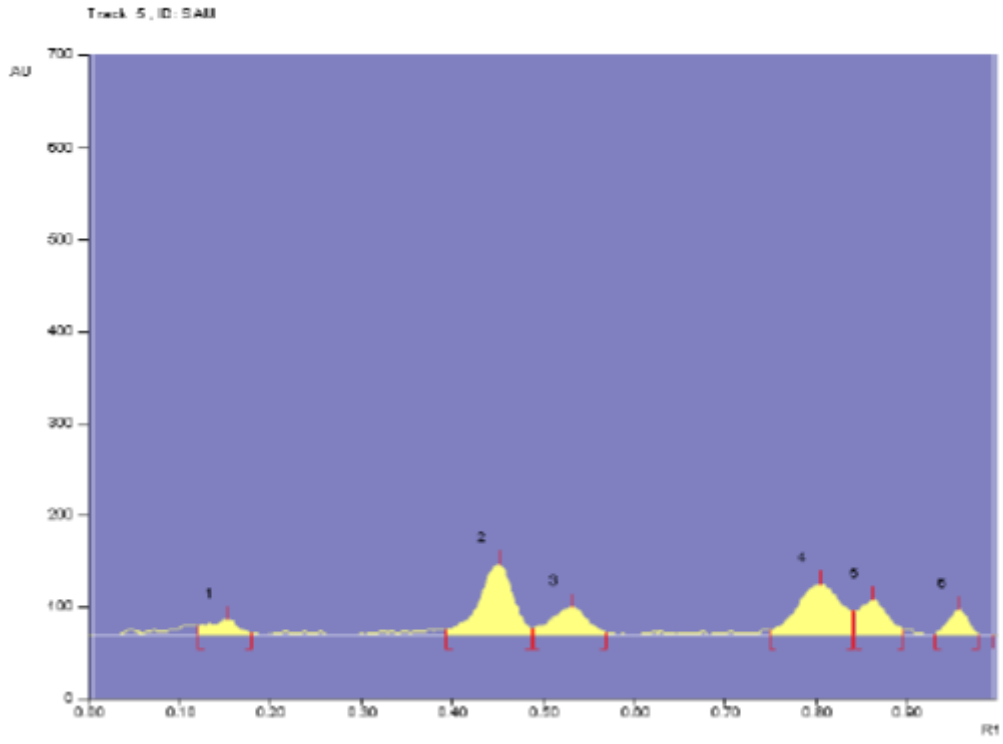


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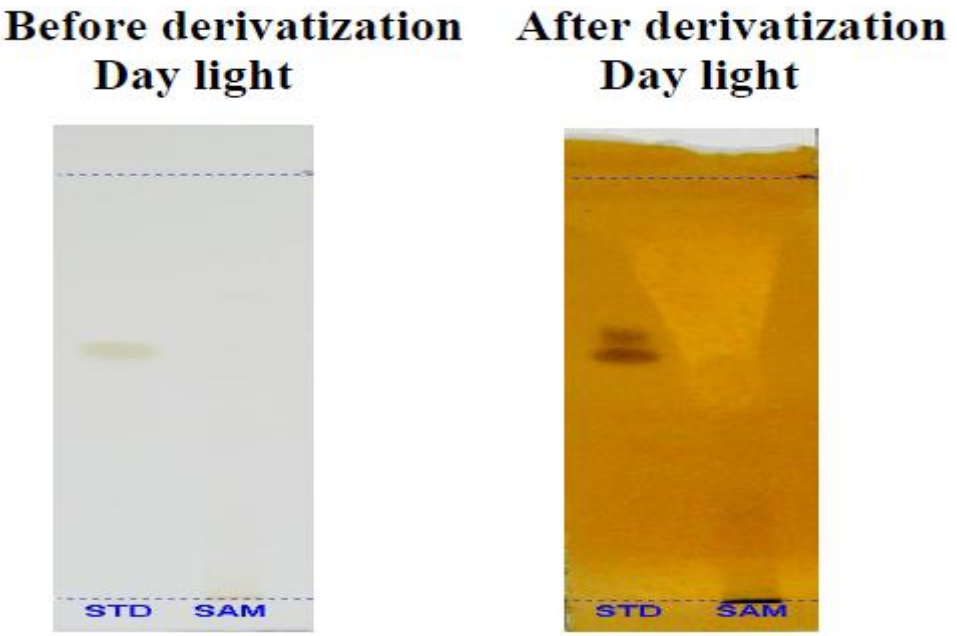
Fig.4a Chromatogram of *Acacia catechu* (L.f) Willd leaf extract for tannins



STD = Standard; SAM = Sample

Fig.4b Densitogram display of *Acacia catechu*(L.f) Willd leaf extract for tannins

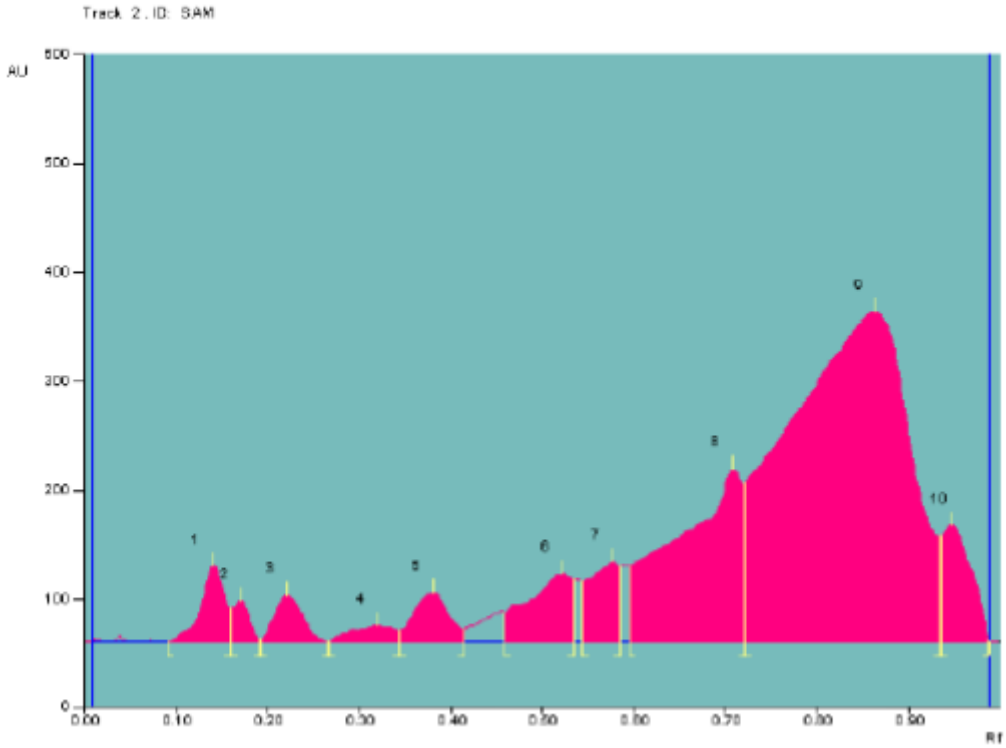
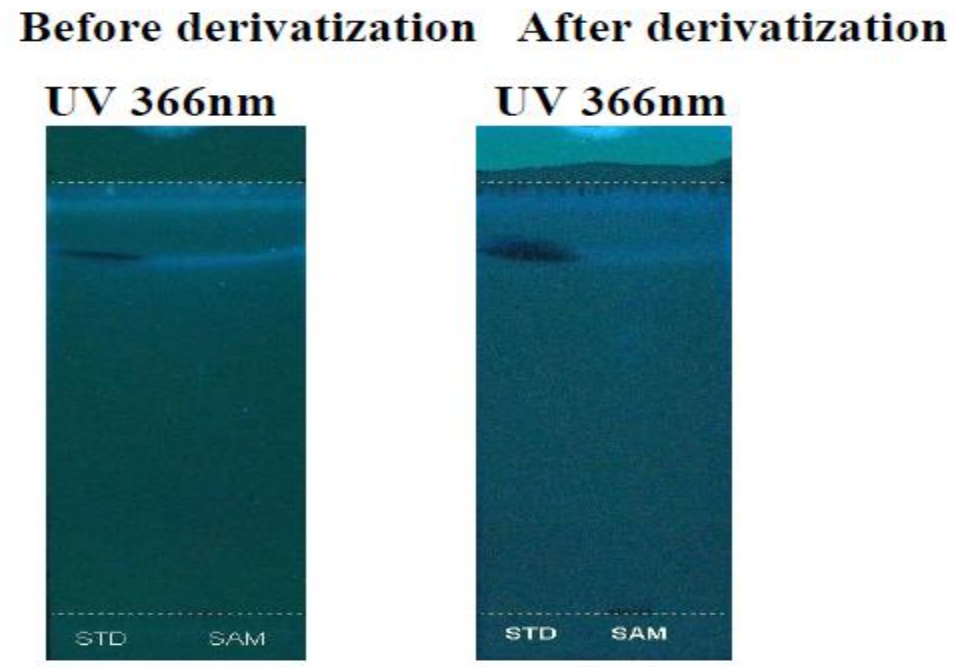


Fig.5a Chromatogram of *Acacia catechu* (L.f) Willd leaf extract for phenolics



STD = Standard; SAM = Sample

Fig.5b Densitogram display of *Acacia catechu* (L.f) Willd leaf extract for phenolics

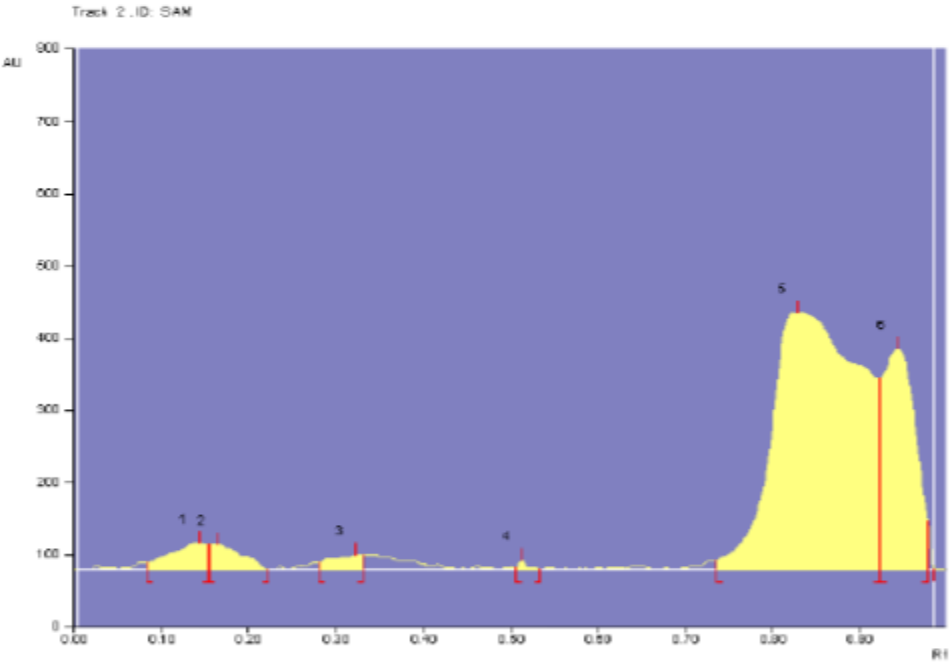
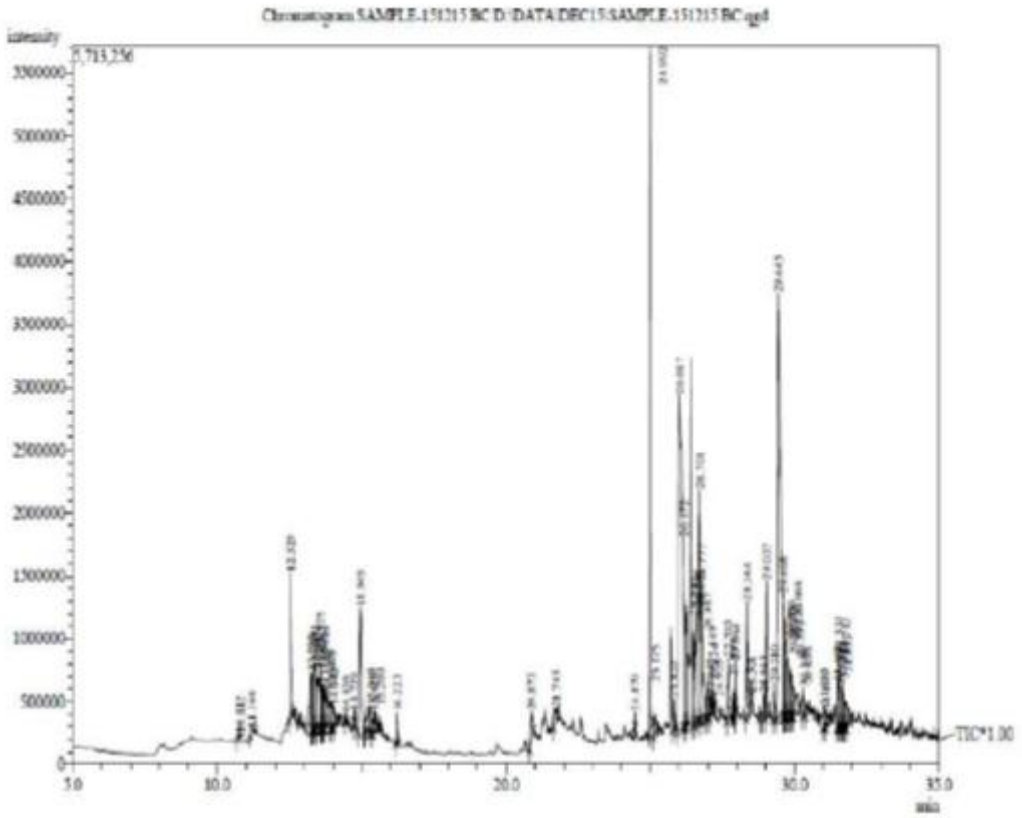


Fig.6 GCMS Chromatogram of *Acacia catechu* (L.f) Willd leaf extract



The Rf value and peak area for standard rutin were found to be 0.51 and 18693.9. The Rf value of 3rd peak (0.53) coincide with the standard. The corresponding densitogram is presented in (figure-3b). Flavonoids are known for their antioxidant, anti-inflammatory and anti-proliferative activities and therefore used in therapeutic roles (Alzand and Mohamed, 2012).

HPTLC fingerprinting profile for tannins

The results from HPTLC finger print scanned at wavelength 500 nm (figure-4a), for tannins revealed 10 polyvalent phyto constituents with the Rf values ranged from 0.14 - 0.95. The component with Rf values of 0.17 and 0.32 were found to be more predominant with area spanning 612.1 and 637.9 respectively. The Rf value and peak area for standard gallic acid were found to be 0.57 and 18001.1. The Rf value of 7th peak (0.58) coincide with the standard. The corresponding densitogram is presented in (figure-4b). Tannins possess antimicrobial, anti-allergic, anti-inflammatory, anticancer and antineoplastic activities (Rievere *et al*., 2009).

HPTLC fingerprinting profile for phenolics

The HPTLC analysis for phenolics recorded in (figure-5a) showed blue brown coloured zones observed at 366nm which revealed the presence of 6 polyvalent phytoconstituents with the Rf values ranged from 0.14 - 0.94. The component with Rf values of 0.17, 0.51 and 0.83 were found to be more predominant with area spanning 1136.92 and 34996.9 respectively. The Rf value and peak area for standard catechol were found to be 0.82 and 45901.2. The Rf value of 5th peak (0.83)

coincide with the standard. The corresponding densitogram is presented in (figure-5b). Phenolic compounds possess biological activities such as anticarcinogen, anti-inflammation, antiapoptosis, antiaging, antiatherosclerosis, cardiovascular protection and cell proliferation activities (Han *et al*., 2007).

GCMS analysis of the plant extract

The GCMS analysis of the plant extract showed 67 peaks (figure-6) that were identified by comparison of the spectra using WILEY and NIST libraries (table-2). The major components in the extract were Benzenamine, 3 ethoxy(1.13%), 12-Methoxy-19-Norpo Docarpa (3.73%), Furo[2,3-d] Pyrimidine-4,6 [5H,7H]-dion(10.20%), 2-Methyl-1,2,3,4-tetrahydro betacarboline (16.26%), 2-Napthalenamine(3.36%), Phthalic acid, 6-ethyl-3-Octyl butyl ester (1.41%), 1- Methoxy-4-(4-Methoxy benzene) (3.23%), Pthalic acid, butyl 2- pentyl ester (4.35%), Hexadecanoic acid, ethyl ester (2.58%), Phytol (1.62%), 9,12,15-Octadecatrienoic acid (2.04%), Butyl-phosphoric acid, di(4-methoxy benzene) (14.82%) and 2-Pyridine carbonitrile, 1,2,5,6. (1.26%).

The phytocomponents with antioxidant activities were found to be 2-Methyl-1,2,3,4 tetrahydro-beta-carboline, 2-Napthalenamine, Hexadecanoic acid, ethyl ester and Phytol. The maximum peak area of 16.26% was observed for 2-Methyl-1,2,3,4 tetrahydro-beta- carboline, that was reported to have potent antioxidant activity (Thomas Herraiz,1999). From this study, it can be concluded that the hydroethanolic leaf extract of *Acacia catechu (L.f) Willd* may serve as a new potential source of medicine as antioxidants due to the presence of various phytochemicals and bioactive compounds, which will be useful to treat various diseases.

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