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Bioassay of tannin rich fraction and identification of compounds using UV-Vis, FTIR and RP-HPLC.

Rajeswari Anburaj[1]* and Vinoth Jothiprakasam[2].

[1]Assistant Professor, MIET Arts and Science College, Department of Microbiology, Affiliated to Bharathidasan University, Tiruchirapalli-620 024.
[2] CAS in Marine Biology, Annamalai University, Parangipettai- 608 502, Tamil Nadu, India

ABSTRACT

This paper represents an analytical approach to characterize tannins and phenolic compounds and to assess the antimicrobial efficacy of the vegetable tannins. Maximum inhibitory effect was recorded in *A. catechu* against *S. aureus* and *S. ficaria*. Characterization by UV, FTIR and Rp-Hplc was done to analyse the vegetable tannins. Existing study depicts that Uv- visible absorption spectrum analysis reveals the presence of condensed tannins, gallotannin (276, 278 nm) and ellagic acid (206-213 nm). Researchers have been used these compounds for different applications such as fungicide, antibiotic and antioxidant The functional assignments indicates the presence of flavonoid and hydrolysable tannins. Rp-Hplc analysis reveals the presence of Gallic acid, catechin derivatives and ellagic acid. *A. catechu* contains higher percentage of gallic acid(75.3%), tannic acid(23.1%) and epicatechin(0.9%), whereas *C. aurantifolia* possess 1-O-Galloyl castalagin(18.5%), catechin(0.9%).

Keywords: Tannin; Antimicrobial screening; Uv-vis; FTIR; RP-HPLC

Corresponding author

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INTRODUCTION

Tannins are the polyphenols widespread in nature, abundant in leaves, fruits and seeds, as well as in wood and bark [1]. They have multiple structure units with phenolic groups and molecular weight ranging from 500 to > 20000 [2]. The main groups of tannins are hydrolyzable tannins and condensed tannins [3]. It is quantitatively important and participates in essential transformations, including photochemical and redox reactions, cation complexation and nitrogen immobilization. It acts as an essential precursor to humic substances and participates in subsequent condensation reactions with proteins and aminoacids by means of quinine formation [4]. When tannic acid substances bind to proteins, it remains toxic towards certain microorganism [5, 6] or metals [7] and their antioxidant nature [8, 9]. They play a major role in the diet of humans and reveal many biologically functions which include protection against oxidative stress and degenerative diseases. These compounds are widely distributed in many plant species and play a major role in protection from predation, act as pesticides, and in plant growth regulation [10]. The tannins (such as condensed tannins) have various therapeutic effects through their antibacterial, antiviral, anti-carcinogenic, anti-inflammatory and antiallergic [11]. The bioactivity capacity of plant is generally depend towards their structure and the degree of polymerization [12]. However, tannins are diverse compounds with great variation in structure and concentration within and among plant species.

This aim of the research work was to isolate the bioassay guided fractions from tannin rich plant material and assessment of compounds by various analytical techniques and to perform antimicrobial assay of compound.

EXPERIMENTAL SECTION

Chemicals

The chemicals used in the study were purchased from Sigma Aldrich. All the solvents ethanol, acetone, ethyl acetate, methanol, acetic acid, silica gel, gallic acid. Nutrient agar, Rose Bengal agar, Sabouraud Dextrose agar and antibiotic discs were purchased from Hi-media. The chemicals and solvents used were of analytical grade.

Plant material

The plant materials such as *Acacia catechu* and *Citrus aurantifolia* were selected for the study. The fruits of *A. catechu* and peel parts of *C. aurantifolia* were collected from in and around Madurai region, Tamilnadu, India. The plants were washed thoroughly, shade dried and homogenized to fine powder using electrical blender and stored in air tight containers.

Extraction of plant

50 g of plant material were packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were of ethanol and acetone. The process of extraction continues for 2 days or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in freezer at 4ºC for their future use.

Column chromatography

The crude phenolic extract (2 g) dissolved in 20 mL of ethanol was applied on a column (2.5 × 60 cm) packed with Sephadex G-25 or G-50 (Sigma-Aldrich Chemical Co.) and eluted with 50% (v/v) acetone. Fractions (4 mL) were collected using a fraction collector. Ethanol (1L), used as first eluent, allowed removing low molecular weight phenolic compounds. Then 600 mL of 50% acetone (v/v) was used to elute tannins. Solvent from tannin fractions was removed using rotary evaporator, and water was removed during lyophilisation.

TLC was conducted using silica gel and 50µl of sample was spotted on plates. The mobile phase used were of ethyl acetate and acetic acid. The plates were sprayed with $Fecl_3$ and brown colour spots were indicated.

Fourier transform Infra red spectroscopy

FT-IR was used to study the functional groups and molecular structure of the extracts. The experiment was determined using Nexus 870 FT-IR instrument. Tannins (0.2 mg) were added into KBr powder (30 mg), mixed and grinded to powder which diameter reached 2 µm, then pressed to a small piece sample in a press machine. Elemental analyses were obtained on a Perkin Elmer 240C microanalyzer.

Identification of phenolics using RP-HPLC

Phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an UV-Vis multiwavelength detector using the same protocol previously described by [11]. Five microgrammes of the extract was diluted in 1 ml of methanol(HPLC grade). The separation was carried out on 250 × 4.6 mm, 4µm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (Solvent A) and water with 0.2% sulphuric acid (Solvent B).The flow rate was kept at 0.5 ml/min. The gradient program was as follows: 15% A/85% B 0 to 12 min, 40% A/60% B 12 to 14 min, 60% A/40% B 14 to 18 min, 80% A/20% B 18 to 20 min, 90% A/10% B 20-24 min, 100% A 24 to 28 min. The injection volume was 20µl and peaks were monitored at 280 nm. Filtration of samples was done through a 0.45µm membrane filter before injection. The experiment was repeated twice and the peaks were identified by congruent retention times compared with standards.

Microorganisms

The microbial cultures of *ATCC*, *Escherichia coli* 433, *Citrobacter freundii* 8128, *Klebsiella pneumonia* 432, *MRSA, Pseudomonas aeuroginosa 1934, Serratia ficaria 8930, Staphylococcus aureus* 1473*, Salmonella typhi* 733*, Aspergillus flavus* 9064*, Aspergillus niger* 10130*, Aspergillus parasiticus* 6777*, Fusarium oxysporum* 4356 and *Fusarium verticilloides* 3322, *Candida albicans 3018* and *Candida glabrata* 3019 were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The bacterial cultures were maintained on nutrient agar slants at 4°C andthe fungal cultures were maintained on potato dextrose broth at 25°C.

Preparation of inoculum

The bacterial cultures were inoculated into nutrient broth and incubated for 24h at 37°C.The growth was compared with 0.5 McFarland; the turbidity of the medium indicates the growth of organisms, while the fungal cultures were inoculated into potato dextrose broth and allowed to incubate at 25°C for 48 h [13].

Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts [14]. The test organism such as bacteria & fungi were respectively lawn cultured on nutrient agar and rose bengal agar by using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork borer. 60µl of the extracts (16 mg/ml) were poured into the well using a sterile micropipette. The plates were incubated at 37°C for 24 hours for bacteria and 25°C at 48 for fungi. After incubation the zone of inhibition was measured by standard scale (Hi-media) in millimetre.

RESULTS AND DISCUSSION

Tannic acid contains secondary metabolites such as polyphenol, and the molecular formula C_{76} H_{52} O_{46}, consisting of a D-glucose to which five gallic acid residues are linked through ester bonds [15, 16]. Ethanol has the capacity to elute phenolics and sugars. Condensed tannins can be eluted by means of acetone-water (1:1; v/v). As a result the high content of phenolic compounds in plant is due to the content of sugars in the crude extract [17]. Tannins or their degradation products are also known to display antimicrobial activity

against a series of human and animal pathogens and exhibit anthelmintic effects against intestinal nematodes [18, 19, 13, 14, 20, 21]. Majority of Gram-positive bacteria are more sensitive to gallotannins than Gram-negative bacteria [22, 23, 24] the outer membrane of gram negative bacteria act as a barrier against hydrophobic and larger hydrophilic compounds, comprise a second mechanism of resistance. The synergistic effects of ellagitannins with antibiotics against antibiotic-resistant bacteria is one of the most noticeable antimicrobial activities of tannins [25]. Corilagin and tellimagrandin I markedly potentiated the activity of β-lactams against methicillin-resistant *Staphylococcus aureus* (MRSA) [26].

Antibacterial Screening

Invitro antimicrobial assay of fractions were represented in Table: 1. *A. catechu* fraction 3 possess maximum inhibition zone of 16.5 mm against *S. aureus* followed by *S. ficaria* (16 mm), *E. coli* & *S. typhii* (15.6 mm), *C. freundii* (15.5 mm), *MRSA* (15.3 mm). Fraction 2 acquired the zone of 16.3 mm against *ATCC* followed by *S. aureus* (15 mm), *S. typhii* (14.6 mm), *E. coli* (14 mm). Significant inhibition was denoted in fraction 1 against *C. freundii* (12.6 mm), *E. coli* and *S. typhii* (12 mm), whereas minimum inhibition was found in fraction 1 (8.6 mm), 2 (11.1 mm), 3 (12.6 mm) against *K. pneumoniae.* Moderate inhibition was recorded in *C. aurantifolia* fraction 1 against *S. ficaria* (13.5 mm) followed by *K. pneumonia* (12.5 mm) and *S. typhii* (12 mm), whereas *MRSA* (8.0 mm) and *P. mirabilis* (8.1 mm) was found to be resistant against fraction 1. *C. aurantifolia* fraction 2 was found to be effective against *C. freundii* (12.3 mm), *MRSA* (11.8 mm) *P. aeuroginosa* & *ATCC* (11.5 mm) whereas least inhibition was denoted in *P. mirabilis* (9.5 mm) & *K. pneumoniae* (8.6 mm).

Table 1: Antimicrobial screening of bioassay guided fractionation of plant samples

	Organism	Plant samples Zone of inhibition in (mm)				
		A. catechu			*C. aurantifolia*	
		1	2	3	1	2
Bacteria	***ATCC***	11.3±0.2	16.3±0.5	14.8±0.7	11.1±0.2	11.5±0.5
	E. coli	12±0.5	14±0	15.6±0.5	9±0	11±1
	C. freundii	12.6±0.5	13.3±0.2	15.5±0	11.6±0.7	12.3±0.2
	K. pnemuoniae	8.6±0.5	11.1±0.2	12.6±0.5	12.5±0.8	8.6±0.2
	MRSA	8.6±0.7	13.1±0.7	15.3±1	8±0	11.8±0.5
	P. mirabilis	8.8±0.5	11.3±0.2	13.5±0	8.1±0.2	9.5±0
	P. aeuro	11.3±0.2	12±0	13.6±0.7	11.3±0.2	11.5±0.5
	S. aureus	10±0.8	15±0.5	16.5±0.5	9.5±0.5	11±0
	S.ficaria	11.6±0.7	12±0.5	16±0	13.5±0	10.6±0.7
	S. typhii	12±0	14.6±0.7	15.6±0.7	12±0	10.8±0.2
Fungi	***A. niger***	5.1±0.7	6.5±0	7.1±0.2	8±0	10±0.5
	A. flavus	7±0.8	8.5±0.5	10±0.5	9.1±0.2	10±0.1
	A.parasiticus	5.1±0.2	8.6±0.7	9.5±0	8.3±0.2	10.3±0.2
	C. albicans	5.3±0.5	7±0	10.3±0.5	14±0.1	15±0
	C. glabrata	5.8±0.7	7±0.8	9.3±0.2	14.4±0.4	15.4±0.4
	F. oxysporum	5.5±0	6.8±0.7	8.6±0.7	12.4±0.4	12.8±0.7
	F. solani	8.5±0.8	9±0	9±0.8	15.1±0.1	16.4±0.3
	F. verticilloides	6.6±0.5	7.6±0.7	8.8±0.2	11.3±0.4	13±0.2

*** Values are mean of ± Standard deviation, n=3**

ATCC- Staphylococcus aureus, *E.coli- Escherichia coli* 433, *C. freundii- Citrobacter freundii* 8128, *K. pneumoniae- Klebsiella pneumonia* 432, *MRSA- Methicillin resistant Staphylococcus aureus, P.aeuroginosa- Pseudomonas aeuroginosa 1934, S. ficaria-Serratia ficaria 8930, S.aureus- Staphylococcus aureus* 1473, *S.typhii- Salmonella typhi* 733, *A.flavus- Aspergillus flavus* 9064, *A. niger- Aspergillus niger* 10130, *A.*

parasiticus- Aspergillus parasiticus 6777, *F. oxysporum- Fusarium oxysporum* 4356, *F. ericilloides- Fusarium verticilloides* 3322, *C. albicans- Candida albicans 3018* and *C. glabrata- Candida glabrata* 3019

Antifungal Screening

Moderate inhibition was found in *A. catechu* fraction 3 against C. *albicans* (10.3 mm) and *A. flavus* (10 mm), *C. glabrata* (9.3 mm) whereas least inhibition was found in *F. oxysporum* (8.6 mm) and *A. niger* (7.1 mm). Fraction 2 possess an inhibition against *F. solani* (9 mm) and *A. parasiticus* (8.6 mm) whereas inhibition was lower in *A. niger* (6.5 mm) and *F. oxysporum* (6.8 mm), Fraction 1 acquired an inhibition in the range of 8.5 mm in *F. solani,* whereas insignificant inhibition was denoted in *A. niger* and *A. parasiticus* (5.1 mm). *F. solani* (16.4 mm) and *C. glabrata* (15.4 mm) remained sensitive towards *C. aurantifolia* fraction 2 followed by *C. albicans* (15 mm) and *F. verticilloides* (13 mm), followed by fraction 1 *C. albicans* (14 mm) and *F. oxysporum* (12.4 mm) Fraction 1 acquired maximum inhibition against *F. solani* (15.1 mm) and *C. glabrata* (14.4 mm) compared to *A. parasiticus* (8.3 mm) and *A. niger* (8.0 mm). *A. flavus* (9.1 mm) and *A. parasiticus* (8.3 mm) were sensitive compared to *C. albicans* and *glabrata*.

In the present study the obtained fractions of three plant samples were analysed by means of UV-Visible spectroscopy, FTIR and Rp-Hplc. The isolation and structure prediction of several tannins, which include hydrolysable, condensed and gallotannins have been elucidated using techniques as UV-visible, Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR), mass spectroscopy [27, 28].

Uv- Visible analysis

Uv- visible analysis of tannin rich fractions were represented in Figure: 1-5. In the UV region (250-2000 nm) the diffuse reflection marks out the 332 and 370 nm bands by structures with extended conjugate; hence the yellowish colour [29]. Proanthocyandins have low molar extinction coefficients and absorption maxima in a region of the UV spectrum (around 280 nm) with many interfering compounds co-eluting [30]. Existing study depicts that the Uv- visible spectrum of *A. catechu* fraction 1- 209 nm, fraction 2- 208.5 nm and fraction-3 was found to be 208.7 cm, showing peak with higher linearity when compared to the fractions of 2 and 3, whereas *C. aurantifolia* fraction 1 was found to have corresponding peaks at 206, 278 nm and fraction 2 peak initiate at the range of 213 nm. *A. catechu* fraction 2 and *C. aurantifolia* fraction 1 represents an strong absorption, an inflection point minimum between 258–259 nm and max between 279–281 nm, confirming the presence of condensed tannins. *A. catechu* fraction 3- 276nm, *C. aurantifolia* fraction 1- 278 nm, represents the characteristic absorption spectrum of gallotannins. The fractions confirm positive results for the presence of ellagic acid from 206 - 213 nm. The UV-VIS range for TA in solution depicts two bands at 213 and 276 nm, both assigned for $\pi \rightarrow \pi^*$ transitions given by aromatic units and C=O groups in UV VIS-region (200-500 nm) [31, 16]. *A. catechu* fraction 1 peaks were emerging at 209, fraction 2 and 3 at 208 nm. Similar peaks were obtained in the fraction 2 having 207 and a slighter discrimination in the range of 272 nm. The initial peak originating in *C. aurantifolia* fractions 1 & 2 were similar in the range of 206 and 213 nm. Therefore, Uv absorption spectrum represents the presence of condensed tannin, gallotannin and ellagic acid.

Fourier transform infra-red spectrophotometer

FTIR analysis of plant tannins were represented in figure: 6-10. Structural elucidation of the tannic acid compounds were analyzed by means of Fourier Transform Infra red spectrometer in the wave number, ranging from 4000 to 400 $^{cm-1}$. According to their structural differences between many species of tannins, some researchers have been used these compounds for different applications such as fungicide, antibiotic and antioxidant [32, 33]. The wide peak in the region 3550–3100 $^{cm-1}$is characteristic of the OH stretching vibration of benzene nucleus and methylol group of tannin [34, 35, 36, 37, 38, 39]. The region of peaks corresponding to the wavenumber 1500-950 $^{cm-1}$ are called fingerprint region for tannins. The peak at 1285 $^{cm-1}$ in the spectrum of pine tannin is a characteristic feature for the flavonoid based tannins [40]. Similar results were correlated with the emergence of peak in *A. catechu* fractions 1, 2. The stretch obtained in the *A. catechu* fraction 1 was 2937 $^{cm-1}$, illustrates that the functional group is Methylene C-H asymmetric , 1638 $^{cm-1}$ having an alkenyl C=C stretch, open-chain imino (-C=N-) group, 1285 $^{cm-1}$ having an primary or secondary, OH in-plane bend, 1874 $^{cm-1}$ belongs to the transition metal carbonyls. The wavenumber 3188 $^{cm-1}$ obtained in fraction 2 suggests that the functional group is an ammonium ion, following wave number 1624 $^{cm-1}$, belongs to alkenyl C=C stretch

whereas 1270 $^{cm-1}$ has an vinylidene C-H in-plane bend. Tannic acid reacts with collagen mainly through hydrogen bonds due to a multitude –OH groups, Tannic acid presents specific bands for –OH associated groups (vOH at 3388 $^{cm-1}$), C=O groups (vC=O at 1715 $^{cm-1}$) and for etheric groups at 1198 – 1025 $^{cm-1}$ in IR region (4000 – 400 $^{cm-1}$) [15, 16]. Therefore it is denoted that the tannic acid contains some aromatic esters due to the signal characteristic bands of carbonyl groups: C=O stretching vibration at 1730-1705 $^{cm-1}$ and C-O at 1100-1300 $^{cm-1}$ [41, 42]. The wave number obtained in fraction 1- 1285 $^{cm-1}$, 2- 1270 $^{cm-1}$ and 3- 1291 $^{cm-1}$suggests that the functional assignment may be alcohols and phenols as it has an C-O stretching. The peaks around 910-740 cm-1 in all spectra's are deformation vibrations of the C-H bond in the benzene rings [35, 38, 39]. In the present study existence of peak in *A. catechu* fraction 1- 812, 2- 811, 3- 814 $^{cm-1}$, *C. aurantifolia* 2-809 $^{cm-1}$.

C. aurantifolia fraction 1- 1238 $^{cm-1}$, 2- 1143 $^{cm-1}$ has similar peaks ranging from 1100-1300 $^{cm-1}$. The fingerprint region of hydrolysable tannins presents an absorption pattern distinct from condensed tannins [43, 44, 45, 40]. In this study, the region 1750–700 $^{cm-1}$ was considered the most informative and carefully examined. *A. catechu* fraction 1 – 812 $^{cm-1}$, 1285 $^{cm-1}$, 1638 $^{cm-1}$ fraction -2 – 811 $^{cm-1}$, 1270 $^{cm-1}$, 1624 $^{cm-1}$, fraction-3 – 814 $^{cm-1}$, 1080 $^{cm-1}$, 1291 $^{cm-1}$, 1628 $^{cm-1}$ represents the absorption pattern ranging from the cited wavenumber. *C. aurantifolia* fraction 1- 795 $^{cm-1}$, 1238 $^{cm-1}$, 1627 $^{cm-1}$ fraction 2- 809 $^{cm-1}$, 1143 $^{cm-1}$, 1628 $^{cm-1}$. Therefore the results convey the presence of hydrolysable tannins in samples. In the spectrum of the untreated nylon 6 fabric, the peaks at 3432, 3401, 3418 and 3419 $^{cm-1}$ confirm the presence of (OH) corresponding to the broad intermolecular hydrogen bonded (OH) between the phenolic hydroxyl groups of tannic acid and carboxyl groups of the dye molecule and dye complex [46]. The functional assignment in *A. catechu* fraction 2- 1624 $^{cm-1}$, 3- 1628 $^{cm-1}$, *C. aurantifolia* fraction 1- 1627 $^{cm-1}$ and 2- 1628 $^{cm-1}$, confirms the presence of amide group (N-C=O) and the stretching vibration of C=O and C-N group. This fact confirms the presence of the electrostatic interaction between the phenolic carboxylic groups of tannic acid and amine groups of Rhodamine B on the treated fabric dyed with the cationic dye solution system. The functional stretches obtained in *A. catechu* fraction suggests superior amount of flavonoid and hydrolysable tannins. The results revealed by spectroscopy indicates the presence of electrovalent, hydrophobic and covalent bonds evidenced by modifications in spectral characteristic absorption bands [16].

RP- HPLC analysis

The tannin fractions were identified by Reverse Phase High Performance Liquid Chromatography. Chromatographic profiles of isolated tannin fractions were represented in figure: 11& 12. The percentage composition of *A. catechu* include gallic acid (75.3%), tannic acid (23.1%), epicatechin (0.9%), B1 dimer (0.19%), vanillic acid (0.19%), carnosic acid (0.09%), catechin (Cya-Cat)(0.09%), hydrolysable tannin (0.08%), ellagitannin (0.07%) coumarine(0.02%), 4β-(2-aminoethylthio) epigallocatechin (0.02%). The complex chromatogram indicates the peaks corresponding to dimer, trimer and tetramer. Condensed tannins are polyphenolic compounds composed of flavan-3-ol sub-units linked mainly through C4–C8 (or C4–C6) bonds [47]. The majority of classes include procyanidins consisting of epicatechin, catechin, and/or their galloylated derivatives, and prodelphinidins consisting of gallocatechin, epigallocatechin and/or their galloylated derivatives. The structural diversity of condensed tannins is due to the different sub-units, interflavonoid bond position, branching and the presence of non-flavonoid substituents such as gallic acid and sugars [48].

The percentage composition of *C. aurantifolia* compounds include gallic acid (47%), trans cinnamic acid (19.2%), 1-O-Galloyl castalagin (18.5%), ellagic acid (19.3%), catechin (18.5%), coumarine (0.9%), castalagin (0.4%), Cya-Cat, 4β-(2-aminoethylthio)catechin (0.9%), B1 dimer (0.4%), epicatechin (0.3%). Ellagitannins are characterised by glucose core esterified with at least one unit of hexahydroxydiphenic acid, which is formed through oxidative coupling between two gallic acid units. Upon hydrolysis, hexahydroxydiphenic acid is released, and spontaneously lactonizes forming ellagic acid [49,50].

The HPLC analysis reveals higher percentage of gallic acid (75.3%), tannic acid (23.1%) and epicatechin (0.9%) in *A. catechu* whereas *C. aurantifolia* possess 1-O-Galloyl castalagin (18.5%), catechin (0.9%) constituent.

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Fig 1: Uv-visible analysis of *A. catechu* fraction-1

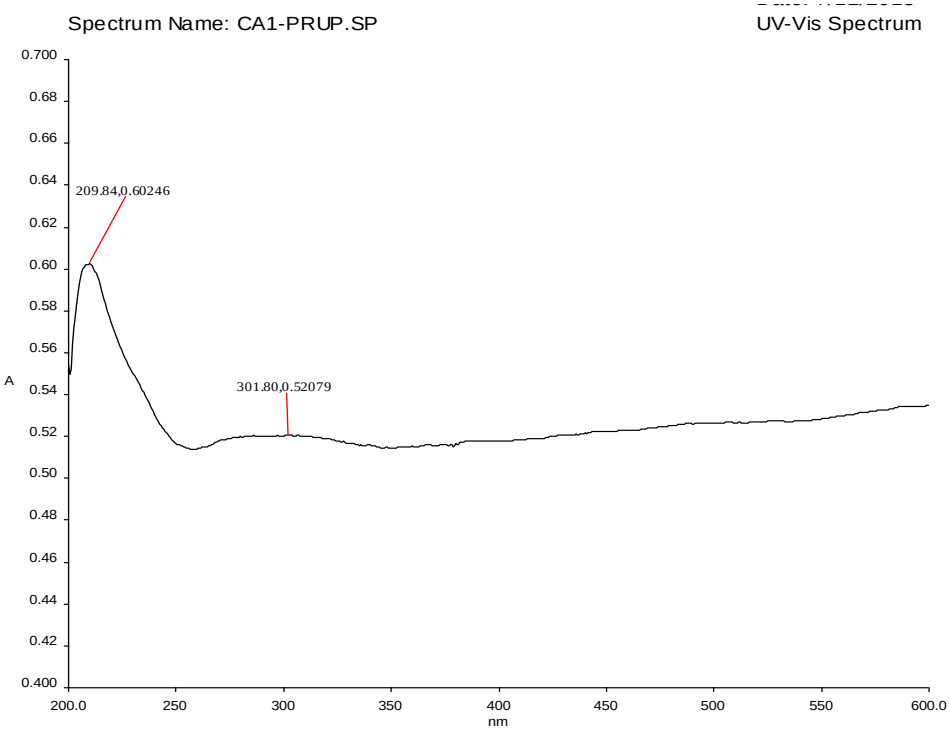

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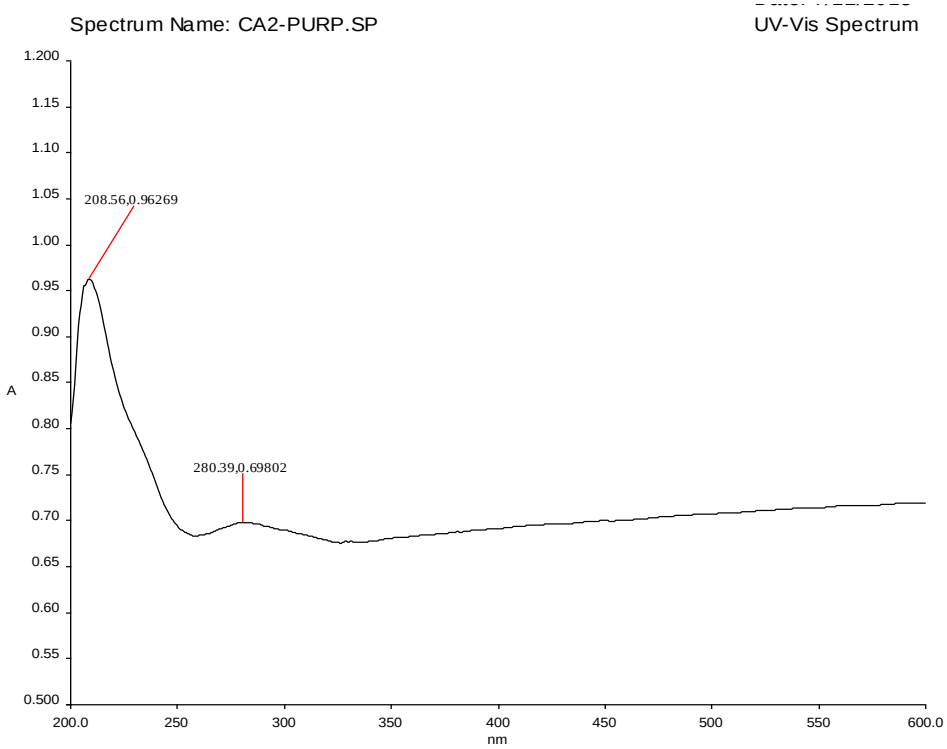
Fig 2: UV visible analysis of *A. catechu* fraction-2



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Scan Speed: 960.00 nm/min

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Fig 3: Uv visible analysis of *A. catechu* fraction-3

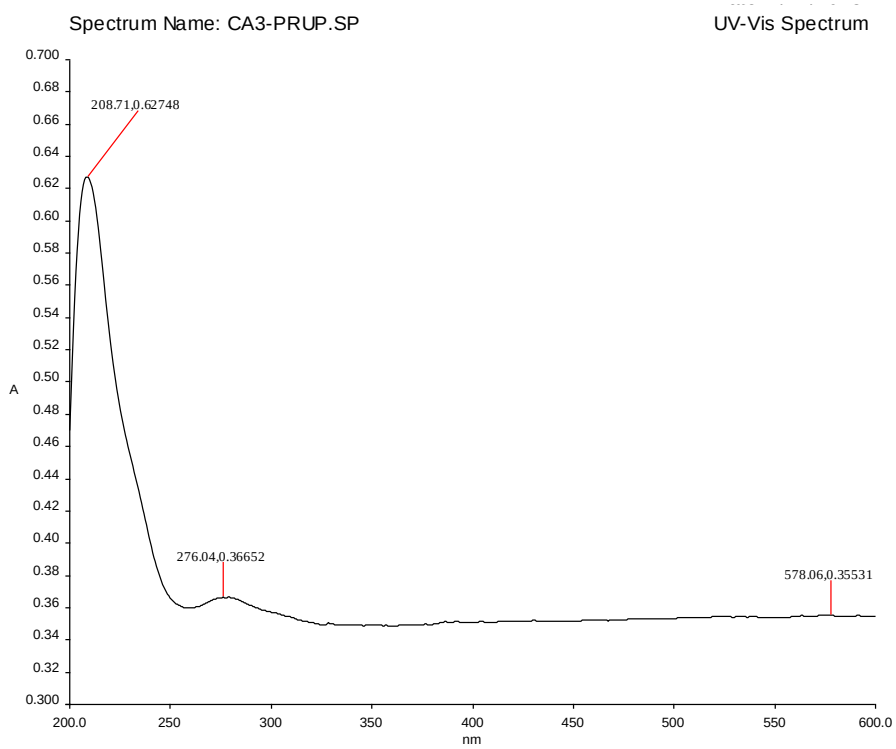

Fig 4: Uv-visible analysis of *C. aurantifolia* fraction-1

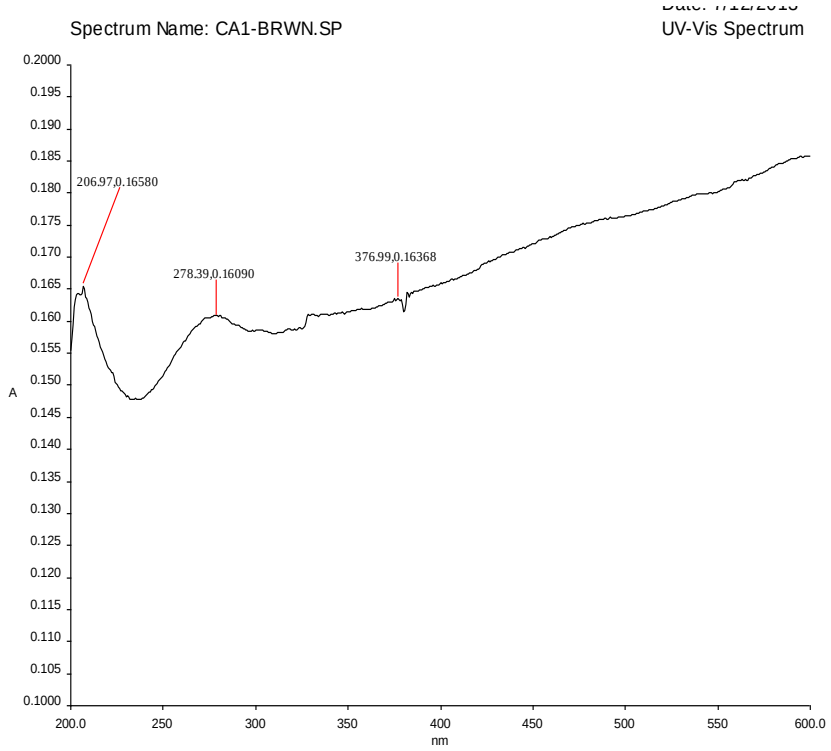

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Fig 5: Uv-Visible analysis of *C. aurantifolia* fraction-2

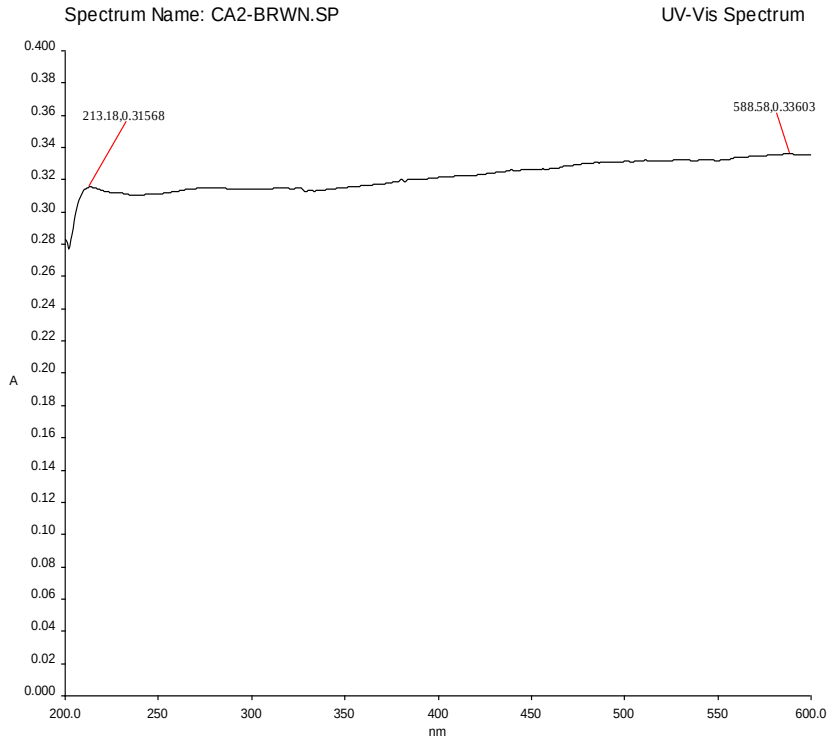
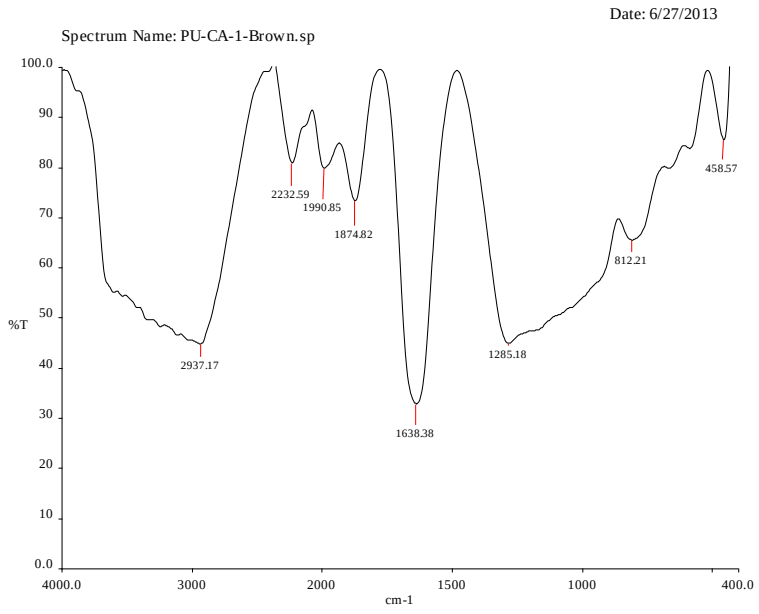


Fig 6: FTIR analysis of *A. catechu* fraction-1



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1285.18 44.96 812.21 65.57 458.57 85.50

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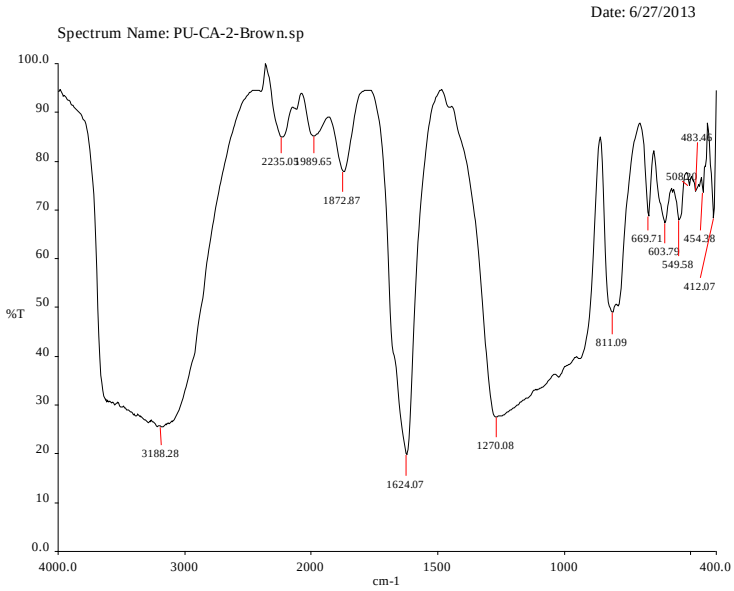
Fig 7: FTIR analysis of *A. catechu* fraction-2



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1270.08 27.50 811.09 49.06 669.71 68.79 603.79 67.37 549.58 68.00
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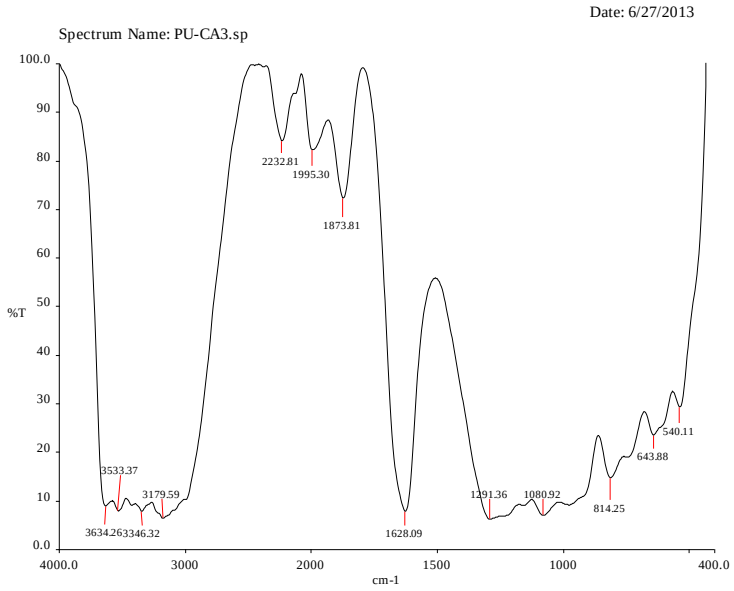
Fig 8: FTIR analysis of *A. catechu* fraction- 3



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PU-CA3.sp 3601 4000.00 400.00 6.29 291.88 4.00 %T 10 1.00

REF 4000 99.99 2000 82.36 600
3634.26 8.98 3533.37 7.91 3346.32 7.90 3179.59 6.46 2232.81 84.16
1995.30 82.28 1873.81 72.36 1628.09 7.92 1291.36 6.29 1080.92 7.09
814.25 14.81 643.88 23.62 540.11 29.32

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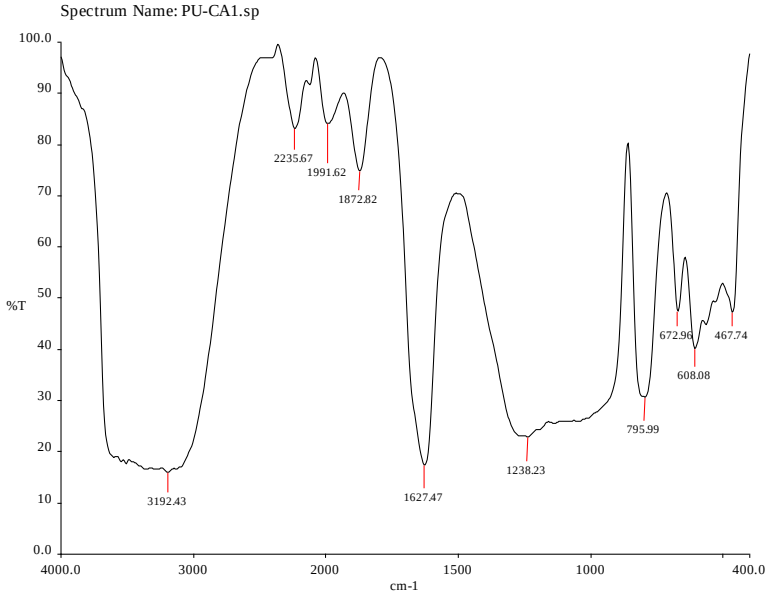
Fig 9: FTIR analysis of *C. aurantifolia* fraction-1

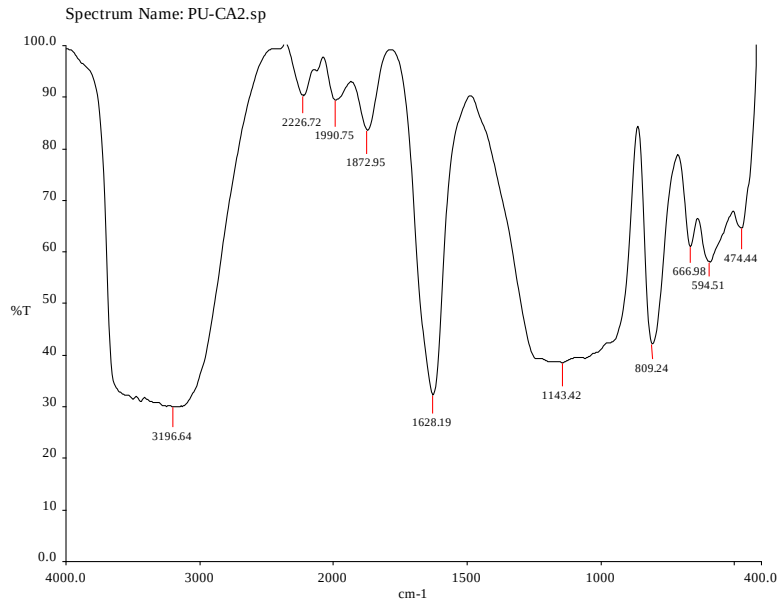


PU-CA1.pk

PU-CA1.sp 3601 4000.00 400.00 15.93 99.69 4.00 %T 10 1.00

REF 4000 97.13 2000 84.35 600
3192.43 15.93 2235.67 83.21 1991.62 84.12 1872.82 74.92 1627.47 17.42
1238.23 22.97 795.99 30.69 672.96 47.48 608.08 40.20 467.74 47.34

Fig 10: FTIR analysis of *C. aurantifolia* fraction-2



PU-CA2.pk

PU-CA2.sp 3601 4000.00 400.00 29.92 172.64 4.00 %T 10 1.00

REF 4000 99.31 2000 89.71 600
3196.64 29.92 2226.72 90.36 1990.75 89.51 1872.95 83.60 1628.19 32.30
1143.42 38.61 809.24 42.13 666.98 61.04 594.51 58.07 474.44 64.64

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Fig 11: Rp-Hplc analysis of *A. Catechu*

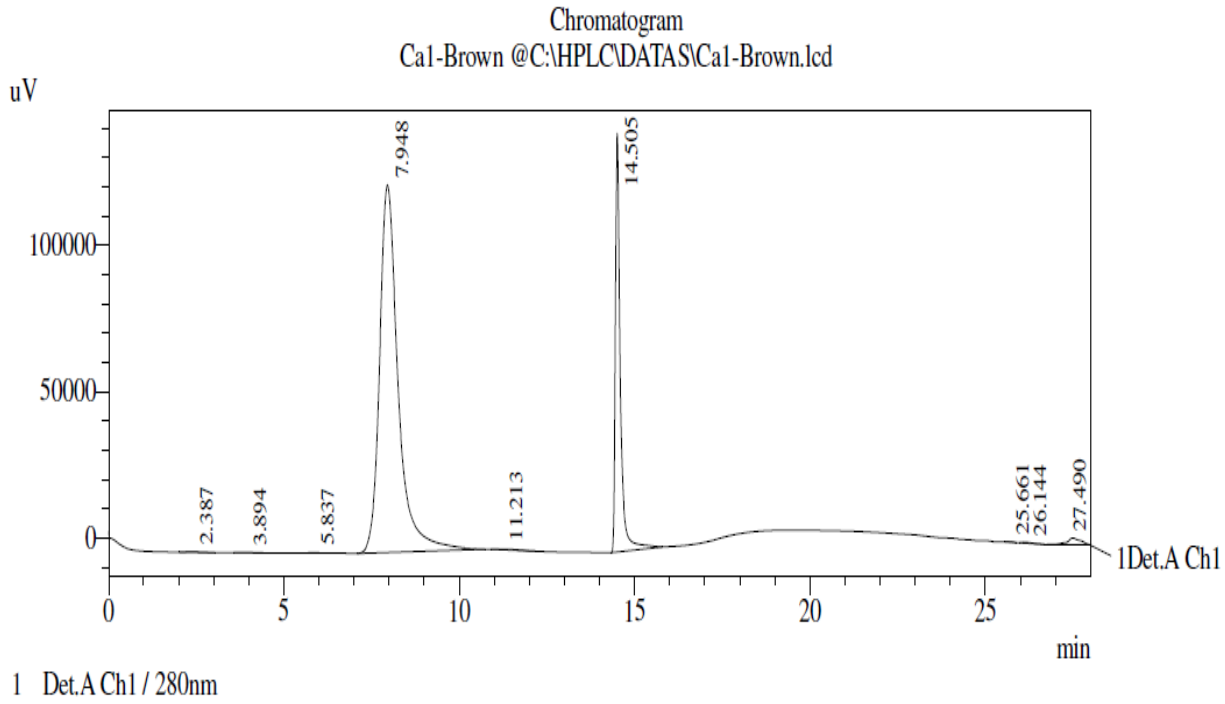
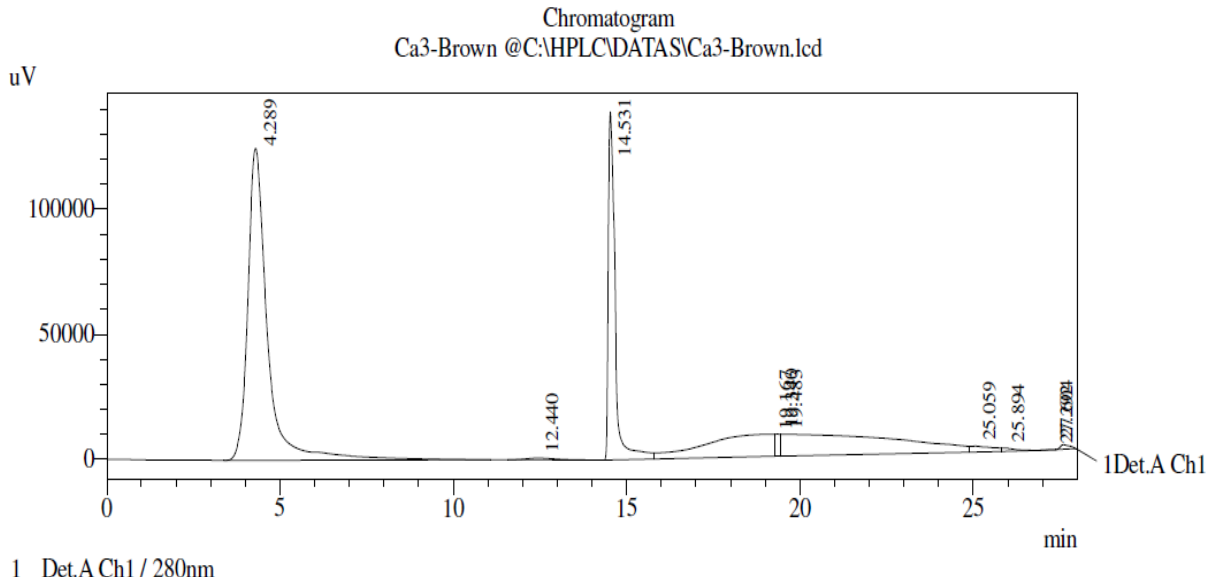


Fig 12: Rp-Hplc analysis of *C. aurantifolia*



CONCLUSION

Therefore, this study highlights the potential of an analytical approach based on spectroscopy analysis to characterize tannins in plant samples. Uv visible absorption spectrum indicates the presence of condensed tannin, gallotannin and ellagic acid, the results revealed by FTIR suggests the presence of hydrolysable and condensed tannins. Flavonoid based tannin was found to be superior in *A. catechu*. The functional assignments indicate that hydrolysable tannin was found in *A. catechu* and *C. aurantifolia.* Hplc analysis reveals the presence of Gallic acid and catechin derivatives.

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