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# CHROMATOGRAPHIC SEPARATION OF TANNIN RICH PLANTS AND CHARACTERIZATION OF COMPOUNDS USING ANALYTICAL TECHNIQUES

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## ABSTRACT

Tannins are polyphenolic compounds found to exhibit antibacterial, antiviral and antioxidant property. This paper represents an analytical approach to characterize tannins, phenolic compounds and to assess the antimicrobial efficacy of the vegetable tannins. Tannins are polyphenol rich plant substances found to possess defensive roles against microbes. The results obtained from antimicrobial screening indicates that *K. pneumonia*e and *C. glabrata* remained sensitive towards *P. niruri* and *T. chebula* fraction, whereas *C. freundii* and *F. solani* was found to be resistant. The Uv- vis spectrum indicates the presence of condensed, gallotannin and ellagic acid. The functional assignments obtained in FTIR analysis indicates the presence of hydrolysable and condensed tannin. Hplc chromatogram reveals the presence of gallotannins such as tetragalloyl glucose, ellagitannin such as pedunculagin and phenols such as gallic acid and catechin. Therefore phenolic hydroxyl groups located on the surface of tannin molecules are believed to participate in biological activities of the tannins.

**KEYWORDS:** Tannin; Antimicrobial assay; phenolic compounds; FTIR; Rp-HPLC.

## INTRODUCTION

Tannins are high molecular weight polyphenolics found in higher plants including many plants used as foods and feed.[1] Tannins are divided into hydrolysable, ellagitannin and condensed tannin. Hydrolysable tannins are a group of gallic acid esters associated with polyols (glucose, glucitol, shikimic acid, quinic acid and quercitol, among others), whereas

the galloyl groups can be crosslinked by oxidation to form complex structures. Condensed tannins or proanthocyanidins consist of coupled flavan-3-ol units that can appear as isolated dimers and compounds with high polymerization grade.[2] The gallotannins (gallic acid esters) are the simplest hydrolysable tannins, were 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (pentagalloyl glucose [PGG]) is the prototype and central compound of the biosynthetic pathway.[3] Ellagitannins are formed by glucose core esterified with at least one unit of hexahydroxydiphenic acid, which is formed by means of oxidative coupling between two gallic acid units. During hydrolysis ellagic acid is formed, whereas hexahydroxydiphenic acid is released. Gallic acid and its derivatives have proven selective antitumor activities, such as: reduction in biochemical markers associated with skin cancer[4]; cell death induction in several cancer cell lines, including leukemia[5,6], murine myeloma[7] and squamous carcinoma.[8] Plant tannins have the ability to react with bacterial cell walls, polysaccharides, carbohydrates and enzymes. They act as defensive compounds that counteract bacteria and fungi by interfering with their surface proteins.[9] Ellagitannins are found to have synergistic action while reacting with antibiotics against antibiotic resistant bacteria.[10]

This present work deals with preparative isolation of tannin rich fractions by Reverse phase High performance liquid chromatography, identification and assessment of antimicrobial efficacy of compounds.

## MATERIALS AND METHODS

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 *Phyllanthus niruri* and *Terminalia bellerica* were selected for the study. The leaves of *P. niruri* and fruit parts of *T. bellerica* 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, and low molecular weight phenolic compounds were removed. Then 600 mL of 50% acetone (v/v) was used to elute tannins. Solvent from tannin fractions was removed by means of rotary evaporator, and water was removed during lyophilisation.

TLC was conducted using silica gel and 50µl of sample was spotted on plates. The mobile phases 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**

Analysis was done by means of Reverse phase High performance liquid chromatography (RP-HPLC) coupled with an UV-Vis multiwavelength detector using the same protocol previously described by Trabelsi.[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 Staphylococcus aureus*, *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**

Nutrient broth was used for inoculation of bacterial cultures 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, whereas the fungal cultures were inoculated into potato dextrose broth and allowed to incubate at 25°C for 48 h.[12]

**Antimicrobial Studies**

The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts.[13] The test organism such as bacteria & fungi were respectively lawn cultured on nutrient agar and rose bengal agar by means of 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 h for fungi. After incubation the zone of inhibition was measured by standard scale (Hi-media) in millimetre.

**RESULTS AND DISCUSSION**

Tannins are plant polyphenols when reacted with protein form stable complexes having molecular weights of 500–3000 kD. The phenolic hydroxyl groups located on the surface of tannin molecules are involved in biological properties of the tannins.[14] According to Amarowicz[15], 80% acetone (v/v) ensured the most complete extraction of phenolic compounds from lentil seeds, especially of flavonols and tannins.

**Antibacterial assay**

*Invitro* antimicrobial assay of fractions were represented in Table: 1. *P. niruri* fraction 3 possess maximum zone of inhibition against *K. pneumoniae* (18.1 mm), *S. aureus* (17 nmm) and *E. coli* (16.6 mm). The zone of inhibition was found to be moderate at *ATCC* (14 mm); whereas minimum inhibition was observed at *C. freundii* (10.3 mm) and MRSA (10.1 mm). Fraction 2 possess maximum zone of inhibition in the range of 16.5 mm against *K. pneumoniae* and *S. aureus*. Moderate zone of inhibition was observed in *ATCC* (13.3 mm) and *S. typhii* (13.1 mm), whereas *C. freundii* and *MRSA* possess minimum zone of inhibition in the range of 9.1 mm. Fraction 1 acquired maximum zone of inhibition against *K. pneumonia* (15.1 mm). Moderate level of inhibition was observed in *E. coli* (13.3 mm) and *P. aeuroginosa* (13 mm); whereas the level of inhibition was found to be minimum at *MRSA* (8.6 mm) and *C. freundii* (8 mm). *K. pneumonia*e remained sensitive towards fractions of *P. niruri*, whereas *C. freundii* remained resistant possessing least inhibition zone.

*T. bellerica* fraction 1 possess maximum zone of inhibition against *K. pneumoniae* (19.5 mm) followed by 18.6 mm against *E. coli* and *P. aeuroginosa*. The zone of inhibition was found to be moderate in fraction 1 (16.8 mm) and 2 (14.8 mm) whereas minimum inhibition was observed in *C. freundii* (11.5 mm, 10 mm) followed by *P. mirabilis* and *S. ficaria* (9.5 mm). Regarding the antimicrobial activity of ellagitannins, punicalagin has been previously reported as efficient agent against *Staphylococcus aureus, Escherichia coli, Aspergillus fumigates, Candida albicans and Cryptococcus neoformans.*[16, 17, 18] The inhibitory spectra of purified gallotannins supports the progress of their application in food products as preservatives.[14]

**Antifungal assay**

*P. niruri* fraction 3 possess maximum zone of inhibition at 16 mm against *C. glabrata*, whereas *F. solani* (10.3 mm) possess minimum inhibition. Fraction 2 (15 mm) acquired maximum inhibition against *C. glabrata* compared to fraction 1 (14.8 mm). Moderate level of inhibition was observed in fraction 3 (12.5 mm), followed by fraction 2 (12.1 mm) and fraction 1 (11.5 mm) against *A. parasiticus*, whereas minimum inhibition was observed at fraction 2 (9.6 mm) and 1(9 mm) against *F. solani*. *T. bellerica* fraction 2 (16.5 mm) and 1 (13.6 mm) possess maximum zone of inhibition against *C. glabrata*, moderate level of inhibition was found at *C. albicans* and *A. flavus* about 12.1 mm, whereas the zone of inhibition was found to be 10.6 mm at *F. oxysporum* and *F. solani*. The results signify that

*C. glabrata* was found to be susceptible towards *P. niruri* fraction whereas *F. oxysporum* and *F. solani* remained resistant. Therefore polyphenol-rich plant extracts often reveal antimicrobial activity[19,20], which allows applications of polyphenolic compounds in food.[21, 22]

**Table: 1 Antimicrobial Screening Of Bioassay Guided Fractionation Of Plant Samples**

| | Organism | Plant samples Zone of inhibition in (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *P. niruri* | | | *T. bellerica* | |
| | | 1 | 2 | 3 | 1 | 2 |
| Bacteria | ***ATCC*** | 11.5±0 | 13.3±0.2 | 14±0 | 10.8±0.28 | 14.8±0.28 |
| | ***E. coli*** | 13.3±0.2 | 15.5±0 | 16.6±0.2 | 17.8±0.28 | 18.6±0.57 |
| | ***C. freundii*** | 8±0 | 9.1±1.2 | 10.3±0.2 | 10±0 | 11.5±0.5 |
| | ***K. pnemuoniae*** | 15.1±0.2 | 16.5±0 | 18.1±0.2 | 16.8±0.28 | 19.5±0.8 |
| | ***MRSA*** | 8.6±0.7 | 9.1±0.2 | 10.1±1 | 9.16±1.05 | 11.1±1.04 |
| | ***P.mirabilis*** | 9.3±0.5 | 10.3±0.2 | 11.3±0.2 | 9.5±0.5 | 12±0 |
| | ***P. aeuro*** | 13±0 | 15.1±0.2 | 16.5±0 | 17.8±0.28 | 18.6±0.5 |
| | ***S. aureus*** | 14.1±0.2 | 16.5±0 | 17±0 | 18.1±0.28 | 19±0 |
| | ***S. ficaria*** | 9.1±0.2 | 9.6±0.5 | 10.8±0.2 | 9.5±0.5 | 12±0 |
| | ***S. typhii*** | 11±0 | 13.1±0.2 | 15.1±0.2 | 15.1±0.28 | 17.8±0.7 |
| Fungi | ***A. niger*** | 10.5±0 | 11±0 | 14±0 | 11.3±0.28 | 13±0 |
| | ***A. flavus*** | 12.1±0.5 | 12.3±0.2 | 13.3±0.2 | 11.6±0.28 | 12.16±0.5 |
| | ***A. parasiticus*** | 11.5±0 | 12.1±0.5 | 12.5±0 | 12.5±0.5 | 13.3±0.3 |
| | ***C. albicans*** | 12±0 | 13.3±0.2 | 14±0 | 12.1±0.28 | 14±0 |
| | ***C. glabrata*** | 14.8±0.5 | 15±0.8 | 16±0.2 | 13.6±0.76 | 16.5±0.5 |
| | ***F. oxysporum*** | 9.8±0.5 | 10.8±0.5 | 11.1±0.2 | 10.6±0.76 | 11.8±0.57 |
| | ***F. solani*** | 9±0.8 | 9.6±0.2 | 10.3±0.2 | 10.6±0.28 | 11±0 |
| | ***F. verticilloides*** | 10.5±0 | 11.6±0.2 | 12.1±0.2 | 11.8±0.57 | 13±0 |

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

**Uv- Visible analysis**

Uv- visible analysis of tannin rich fractions were represented in Figure: 1-5. UV- visible spectrum was carried out using Cary 100 Perkin-Elmer UV–visible spectrophotometer. The data obtained in this study shows the existence of characteristic Uv absorption pattern for different types of tannins analysed. Uv spectrum for tannic acid in solution presents band at 207- 284 nm assigned for π-π * transitions specified by aromatic units and C=O groups in Uv- vis spectrum (200-500 nm).[23] Similar results were observed in *P. niruri* fraction 1 & 2 presenting a strong absorption pattern at 208 nm, whereas in fraction 3 the peak originated at 207 nm. The peaks produced at *P. niruri* fraction 1 (274 nm) and 3(276 nm) indicating the presence of gallotannin. *P. niruri* fraction 1(377 nm) indicates the degradation process of valonia tannin. The peak at *P. niruri* fraction 1(274 nm) and 2(276 nm) indicates the presence

of gallic acid. The peak at 213- 278 nm indicates the presence of condensed tannins, similar results were observed in *P. niruri* and *T. bellerica* fraction. The peak originating at 216 and 219 nm in *T. bellerica* fraction 1 & 2 indicates the presence of aromatic and C=O groups. The Uv-vis region around 260-280 nm indicates the presence of condensed and gallotannin. The peak at 278 nm in *T. bellerica* fraction 1 and 2 indicates the presence of condensed and gallotannin. The peak at 579 nm indicates the presence of gallic acid and catechin in *T. bellerica* fraction 2. Ellagic acid, the monomer of ellagitannins, produce absorption peaks around 200 nm with two max at 255 and 365 nm. These wavenumber indicate the low molecular weight fraction of hydrolysable tannin .[24] Moreover, the n-pi * band from the 380 – 515 nm domain points out of to the presence of extended conjugation structures; denoting the presence of extended conjugation structures in *oak* and *quebracho* due to the gallic acid (in *oak*) and to the catechins with a greater molecular mass (in *quebracho*).[25]

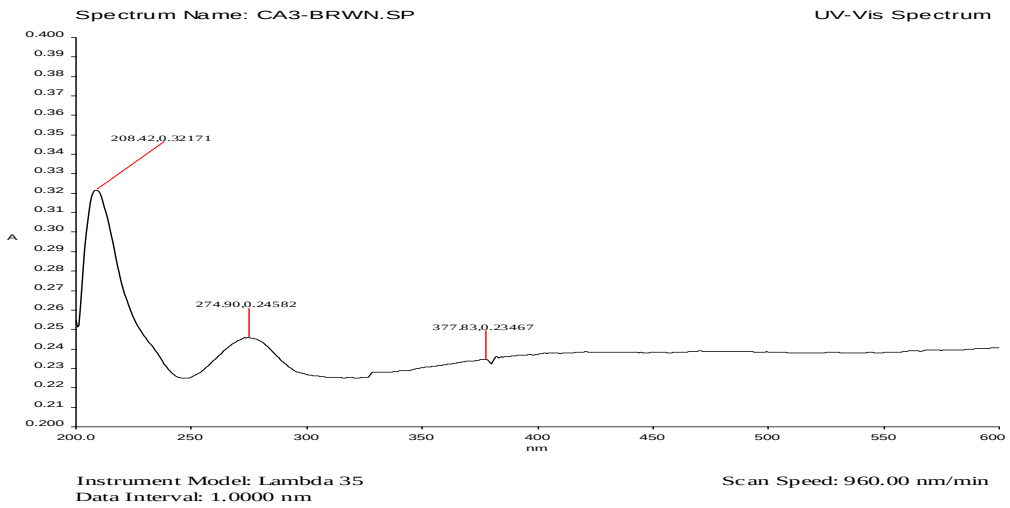



**Fig: 1 Uv- visible analysis of *P. niruri* fraction 1**

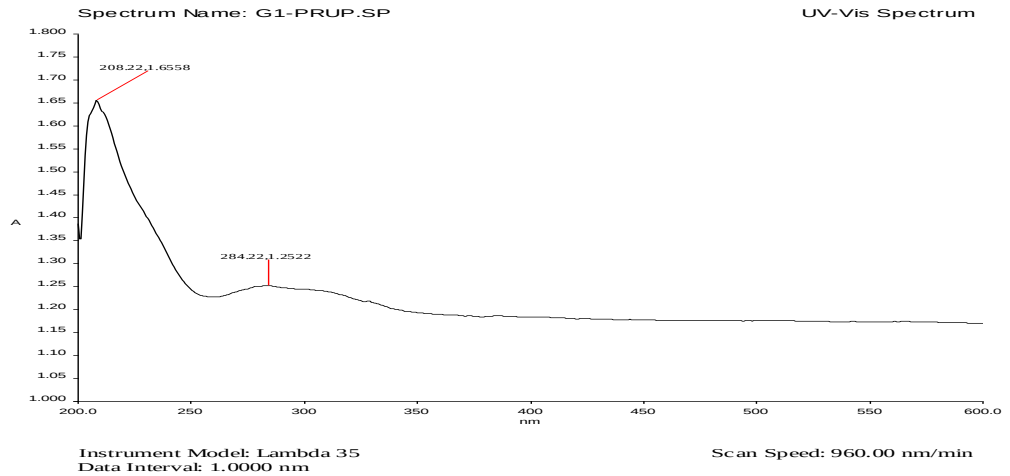



**Fig: 2 Uv- visble analysis of *P. niruri* fraction 2**

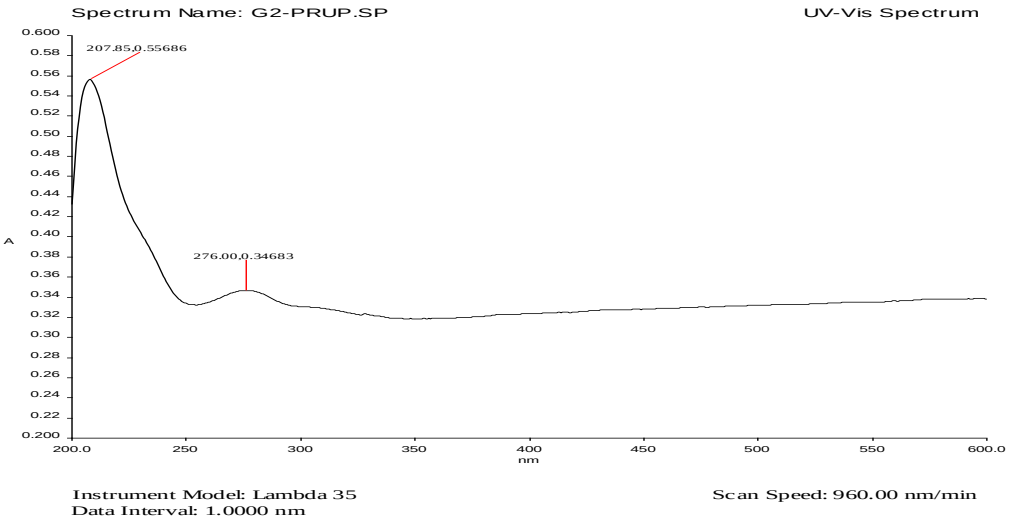



**Fig: 3 Uv- visble analysis of *P. niruri* fraction 3**

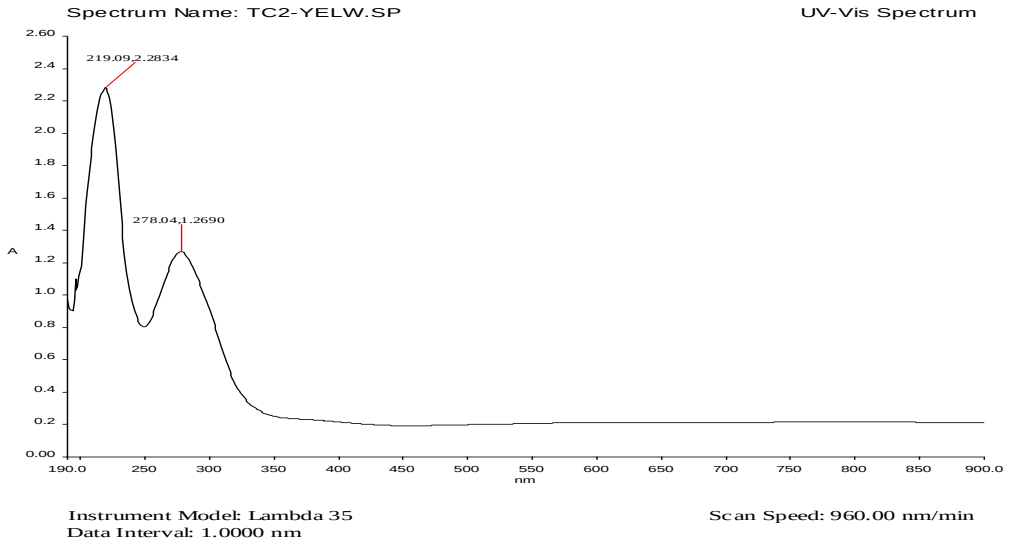



**Fig: 4 Uv- visible analysis of *T. bellerica* fraction 1**

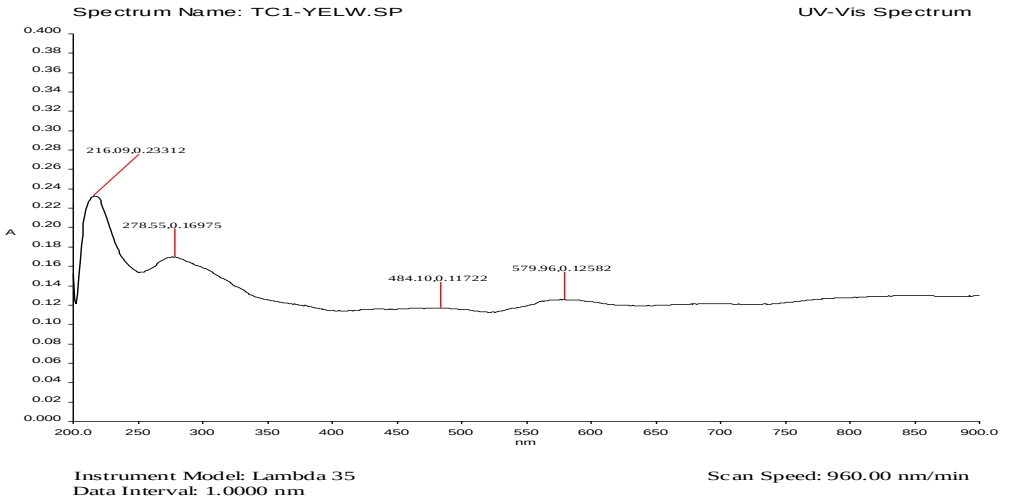


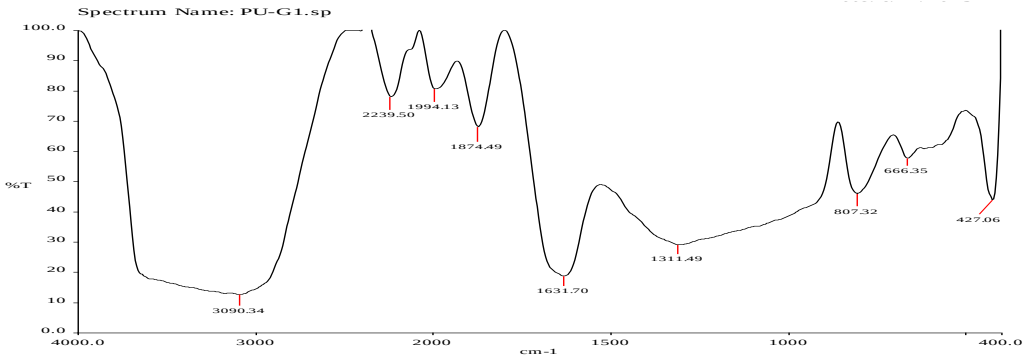

**Fig: 5 Uv- visible analysis of *T. bellerica* fraction 2**

**Fourier transform infra-red spectrophotometer**

FTIR analysis of plant tannins were represented in figure: 6-10. The extensive peak in the region 3550–3100 $^{cm-1}$ is characteristic feature of the OH stretching vibration of benzene nucleus and methylol group of tannin.[26, 27, 28, 29, 30, 31] The wavenumber obtained in *P. niruri* fraction 2(3413.93 $^{cm-1}$, 3330.30 $^{cm-1}$) and fraction 3 (3543.90 $^{cm-1}$, 3292.35 $^{cm-1}$) correlates with previous results. The region of peaks 1500-950$^{cm-1}$ are called fingerprint region for FTIR spectra of tannins. The functional assignment of *P. niruri* fraction 1 (1311.49 $^{cm-1}$), 2 (1236.22 $^{cm-1}$, 1132.11 $^{cm-1}$, 1026.55 $^{cm-1}$, 1080.08 $^{cm-1}$) and 3(1213.47 $^{cm-1}$, 1093.35 $^{cm-1}$, 1028.01 $^{cm-1}$) indicates the finger print region of tannins. The wavenumber obtained at fraction 2 (1873 $^{cm-1}$) belongs to the transition metal carbonyls, following wave number 1627 $^{cm-1}$ belongs to alkenyl C=C stretch, fraction 3(1630.57 $^{cm-1}$) having an alkenyl C=C stretch, open-chain imino (-C=N-) group. Tannic acid reacts with collagen mainly through hydrogen bonds due to a multitude –OH groups, The functional assignment presents definite bands for –OH associated groups (νOH at 3388 $^{cm-1}$), C=O groups (νC=O at 1715 $^{cm-1}$) and for etheric groups at 1198 – 1025 $^{cm-1}$.[32,33] The results were corraborated with the functional assignments obtained at fraction 2 (1132.11 $^{cm-1}$, 1080.08 $^{cm-1}$) and 3(1093.35 $^{cm-1}$, 1028.01 $^{cm-1}$). 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 $^{cm-1}$.[34, 35] The wave number obtained in fraction 2- 1236.22 $^{cm-1}$, 1132.1$^{cm-1}$ and fraction 3- 1213.47 $^{cm-1}$ indicates the presence of carbonyl groups.

The extensive peak obtained in *T. bellerica* fraction 1(3408.48 $^{cm-1}$) and 2(3408.82 $^{cm-1}$) indicates the presence of methyloyl group of tannin.[26, 27, 28, 29, 30, 31] The functional stretches obtained at fraction 1 (802.68 $^{cm-1}$) and 2 (800.71 $^{cm-1}$) are known as finger print region of hydrolysable tannin. The functional stretches at fraction 1(1623.55 $^{cm-1}$) and 2(1624.23 $^{cm-1}$) belongs to alkenyl C=C stretch. The spectrum obtained at fraction 1 (3408.48 $^{cm-1}$) and 2(3408.82 $^{cm-1}$) confirms the presence of (OH) corresponding to the broad intermolecular hydrogen bonded (OH) between the phenolic hydroxyl groups of tannic acid and carboxyl groups.[36] The wavenumber obtained at 1110.11 $^{cm-1}$, 1110.84 $^{cm-1}$ indicates the presence of condensed tannin, whereas the stretch obtained at 1080 $^{cm-1}$ indicates the presence of gallotannin. The region of peaks at fraction 1 (1110.11 $^{cm-1}$) and 2 (1110.84 $^{cm-1}$) reveals the presence of carbonyl and etheric groups.[32,33] The peaks at 1,620 – 1,636 $^{cm-1}$ represent carbonyl groups from polyphenols such as epicatechin gallate(ECG), epigallocatechin gallate(EGCG), catechin gallate(CG), epigallocatechin(EGC), and theaflavin.[37] Similar

results were observed in *P. niruri* fraction 1 -1631.70 $^{cm-1}$, 2- 1627.25 $^{cm-1}$, 3- 1630.57 $^{cm-1}$ and *T. bellerica* fraction 1-1623.55 $^{cm-1}$, 2-1624.23 $^{cm-1}$.

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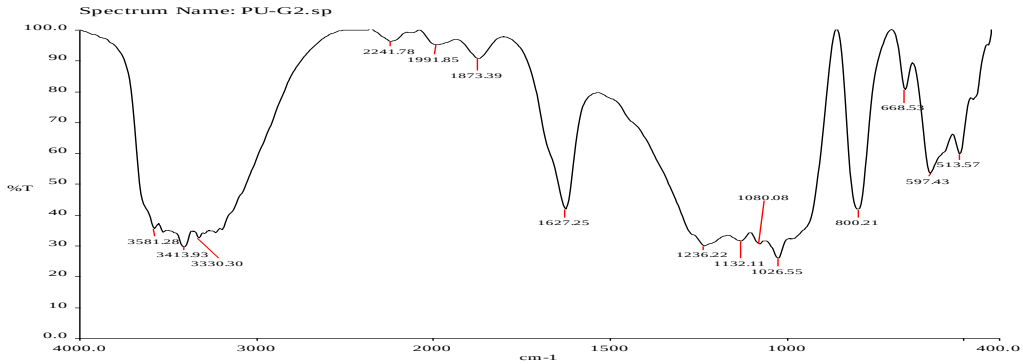
1311.49 29.24 807.32 46.12 666.35 57.76 427.06 44.00

**Fig: 6 FTIR analysis of *P. niruri* fraction-1**

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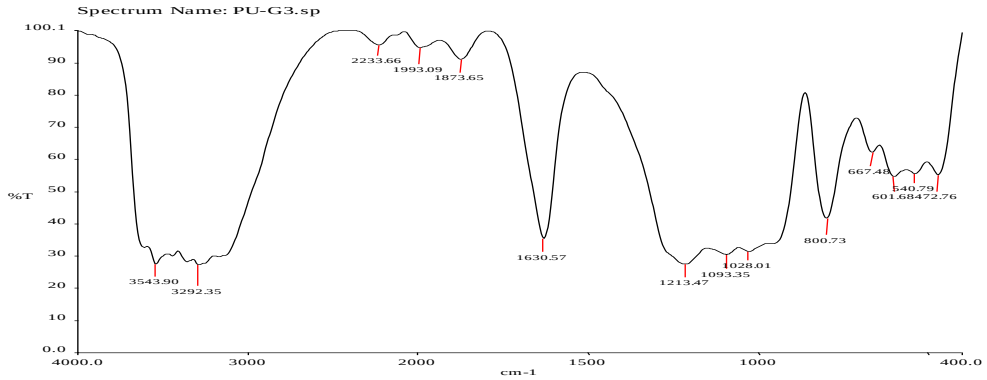
1873.39 90.85 1627.25 42.07 1236.22 30.07 1132.11 31.59 1080.08 30.62
1026.55 26.07 800.21 41.88 668.53 80.69 597.43 53.61 513.57 59.79

**Fig: 7 FTIR analysis of *P. niruri* fraction-2**

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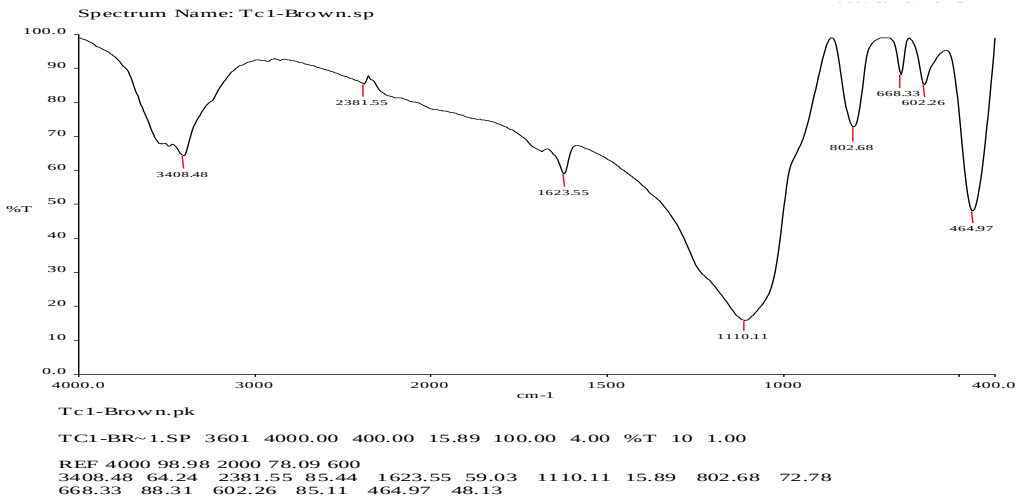
1630.57 35.60 1213.47 27.61 1093.35 30.52 1028.01 31.46 800.73 41.74
667.48 62.28 601.68 54.77 540.79 55.65 472.76 55.30

**Fig: 8 FTIR analysis of *P. niruri* fraction-3**




Tc1-Brown.pk

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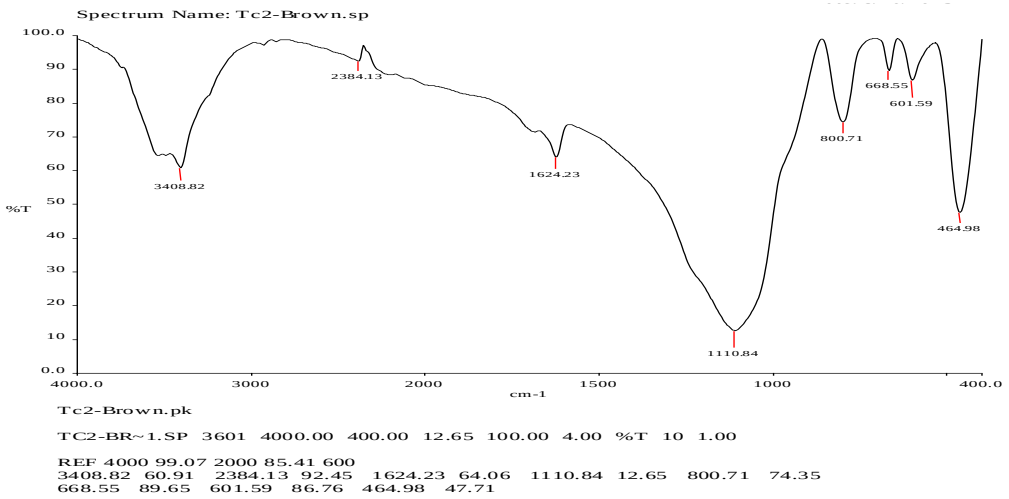
668.33 88.31 602.26 85.11 464.97 48.13

**Fig: 9 FTIR analysis of *T. bellerica* fraction-1**

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668.55 89.65 601.59 86.76 464.98 47.71

**Fig: 10 FTIR analysis of *T. bellerica* fraction-2**

**Rp- Hplc analysis:** The tannin fractions identified by Reverse phase high performance liquid chromatography were represented in figure: 11-13. Analysis of proanthocyanidins is frequently done by means of reverse-phase high-performance liquid chromatography. Applications of reversed-phase HPLC analysis of proanthocyanidins can be found in a wide variety of foods. For example, C18 columns have been employed mostly to determine the monomeric and dimeric procyanidins in grape juice and red wine.[38, 39]

The percentage chromatogram of *P. niruri* fraction 1 include 47.9% epigallocatechin gallate and gallic acid, 17.4% –epicatechin 3-O- gallate and phloroglucinol adduct of extension subunit, P.coumaric acid, 16.1%- trans cinnamic acid, tetraglloyl glucose, 15.7%- epicatechin 3-O-gallate, 15.7%- quercetin dehydrate, 0.09% - caffeic acid, 0.28%- catechin- 0.21%- 3,4 dihydroxyphenylacetic acid, 0.2%- tetramer. The percentage composition of *P. niruri* fraction

2 include 41%- pedunculagin, gallic acid, 20.5%- coumaric acid, 20.5%- quercetin dehydrate, tetragalloyl glucose, 19.9%- epicatechin 3-O gallate and phloroglucinol adduct of extension subunit, 0.8%-trans hydroxyl cinnamic acid, 0.2%- trans cinnamic acid, 0.17%- flavones.

The percentage compostion of *T. bellerica* include 61.11%- gallic acid, epigallocatechin, 37.1%- epicatechin 3-O gallate, 1%-flavones, 0.3% -epigallocatechin gallate, catechin and pedunculagin, 0.1%-caffeic acid, 0.1%-epicatechin, 0.03%-trans cinnamic acid, 0.1%-apigenin. Catechin/epicatechin was the basic units occurring in the condensed tannins, and A-type and B-type linkages were most common among the structural units of polymers.[40, 41] EA has also shown antioxidant activity as an inhibitor of in vitro lipid peroxidation and, because of its combined actions, it is used in the food industry [42].

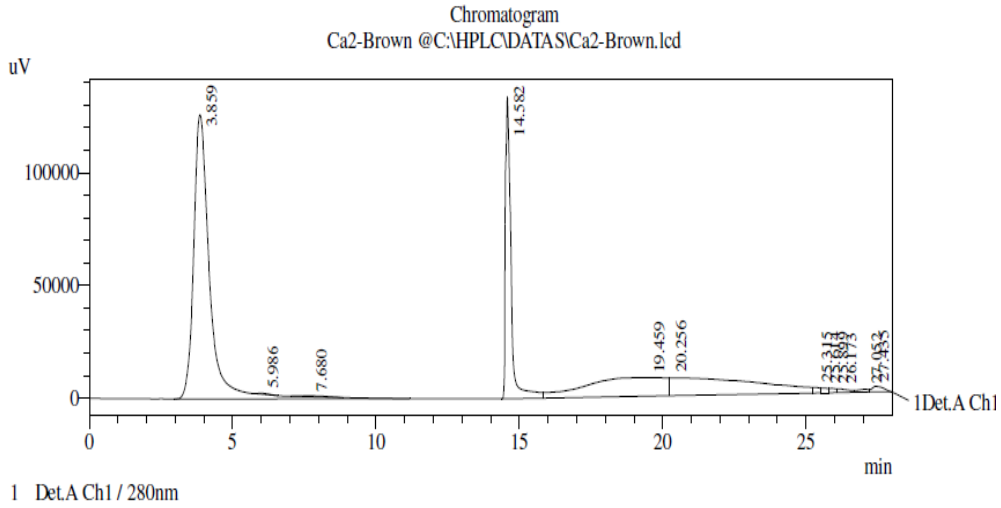


**Fig: 11 Rp-Hplc analysis of *P. niruri* fraction-1**

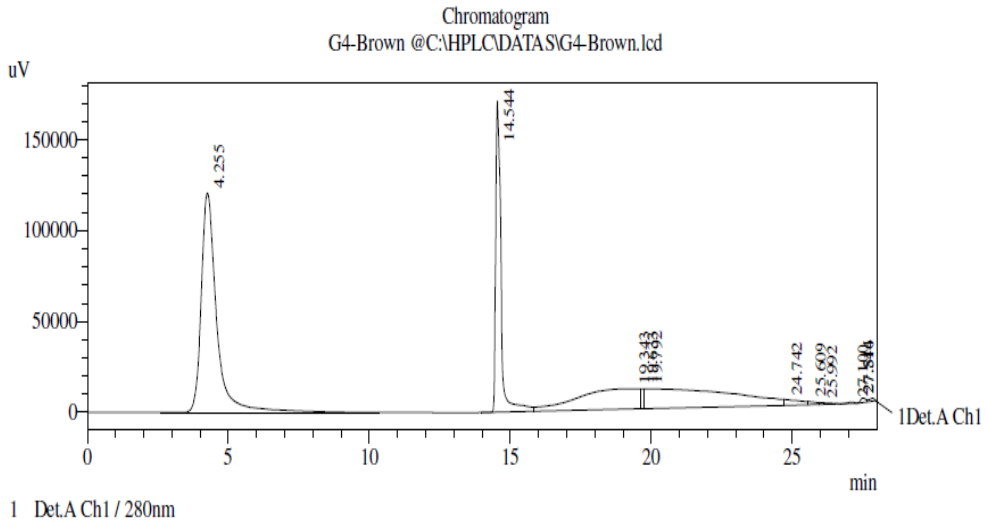


**Fig: 12 Rp-Hplc analysis of *P. niruri* fraction-2**

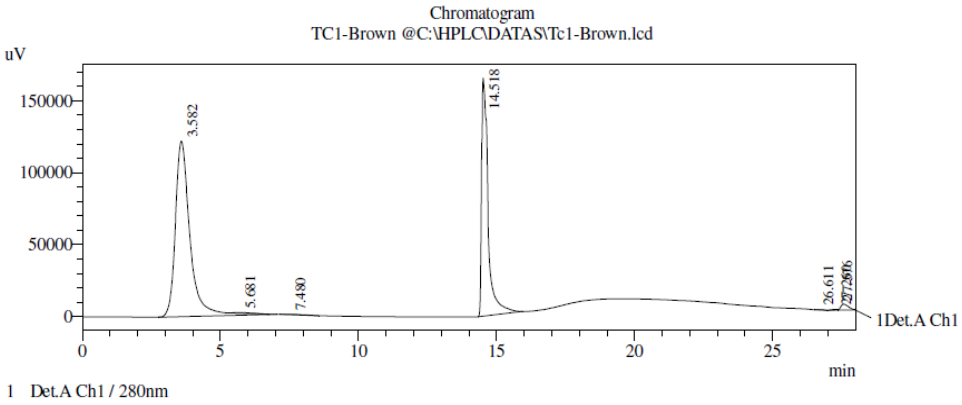


**Fig: 13 Rp-Hplc analysis of *T.bellerica* fraction-1**

## CONCLUSION

Tannins are complex polyphenols synthesized by a wide range of plants, they are proposed to play key roles in the chemical defences of the plant species. Hydrolysable tannin and condensed tannin were found in plants of *P. niruri* and *T. bellerica*. The results obtained from antimicrobial assay indicates that *K. pneumoniae* (19.5 mm) and *C. glabrata* (16.5 mm) remained sensitive towards *P. niruri* and *T. chebula* fraction, whereas *C. freundii* (8 mm) and *F. solani* (9 mm) was found to be resistant. The existence of peak at 260-280 nm indicates the presence of condensed and gallotannin. The functional assignment in *P. niruri* fraction 1 - 1631.70 $^{cm-1}$, 2- 1627.25 $^{cm-1}$, 3- 1630.57 $^{cm-1}$ and *T. bellerica* fraction 1-1623.55 $^{cm-1}$, 2-1624.23 $^{cm-1}$ indicate the presence of polyphenols such as catechin gallate, epicatechin gallate, epigallocatechin, epigallocatechin gallate, and theaflavin. The developed HPLC technique is precise, specific, accurate and robust for the determination of phenolic compounds. *T. bellerica* possess higher percentage of epicatechin 3-O gallate and gallic acid compared to *P. niruri*. The tannin rich plant materials possess gallotannins such as tetragalloyl glucose, ellagitannin such as pedunculagin and phenols such as gallic acid and catechin. Therefore the proposed method can be used for qualitative as well as quantitative analysis of gallic acid.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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