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By using RP-HPLC Technique, Quantitative and Qualitative analysis of Gallic acid from Industrial waste

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Abstract
RP- HPLC is a simple, precise and simple technique which was used for qualitative assurance of Gallic acid from testa of *Anacardium occidentale L.*, UV detection at 271nm was developed and validated. Separation was performed on a Phenomenax column (150 × 4.6 mm, 5 µm pore size) by using mobile phase, 80:20 ratiog of acetonitrile and water containing 0.01% v/v ortho phosphoric acid for the present study. For the separation and identification of gallic acid direct injection method has been performed in the HPLC technique. Studies, which activated the accurateness of the method, 99.63% recovery percentage was showed for this acid.

Keywords: tannin, gallic acid, *anacardium occidentale l*, testa

1. Introduction
All most in all plants gallic acid is present. Gallic acid is beginning in about all plants. A powerful well known antioxidant, Gallic acid is found in variety of foods and herbs. Herbs such as blueberiies, walnuts, flax seed, tea and apples all contain gallic acid. It is also present in sumac, gallnuts, hazel, watercress, oak bark and other plants. Gallic acid (3,4,5-trihydroxy benzoic acid) is a phenolic compound and finds application in various fields. In the higher plants, the sysnthesis of gallic acid is still an open question. An α-oxidation of 3,4,5-trihydroxycinnamic acid and, by hydroxylation of 3,4-dixydroxycinnamic (protocatechuic) acid, and (**c**) by direct dehydration of 3-dehydroshikimic acid, an intermediate compound of the shikimate pathway is the three pathways were proposed for accumulation of gallic acid. An antibacterial agent in combination with sulfonamide is a lot of important use in for accomplishment trimethoprim (TMP), Gallic acid is a poly-phenolic compound, which is obtained from the hydrolysis of natural plant poly-phenols and used as a reductant. It has been used historically to yield blue ink as its reduction of iron chloride produces a blue precipitate. By the enzymatic hydrolysis of tannic acid, Gallic acid (3, 4, 5-hydroxy benzoic acid) is produced at the industrial scale level. Anti-malarial drug Trimethoprim is manufactured by using Gallic acid. An antioxidant propyl gallate, used in food industry was also manufactured by using gallic acid. Gallic acid is also used to produce Pyrogallal, which is used in leather and hair, staining fur and also a photographic developer. Further, gallic acid possesses numerous biological activities such as antiviral, antibacterial, anti- poptotic and analgesic activities. Several different biological backdrop and bartering applications, gallic acid is an admixture of abundant absorption to both biologic and actinic industries. Present paper reports, a simple, accelerated and absolute acclivity HPLC method with an economical mobile phase for quantification and qualification of gallic acid.

2. Plant material
Cashew (Anacardium occidentale L.) testa were acclimated as substrate in the solid state fermentation. Tannins from testa were analysed.

Fermentation and isolation of tannase
For one week at thirty degree centigrade for week, *A. Niger* changed into grown of changed czapek's dox medium. Spores had been calm beneath antibacterial action utility Tween eighty. The organized spore suspension changed into adjusted to 107 spores/ml. 250ml Erlenmeyer flasks containing 50g of cashew testa, 3g tannic acid and KH2PO4 5g. NH4NO3 10g, MgSO47H2O 1g, CaCl26H2O 0.1g, MnCl26H2O 0.02g, NaMo O4 2H2O 0.01g, FeSO47H2O 0.125g, turned into adjusted to pH 5.5 with 100mM NaOH and incubated at thirty degree centigrade for 2 days with three milliliters of organized spore suspension inoculum. The brewed biomass alloyed very well observed via centrifugation at ten thousand revolutions according to minute to abolish the mycelia mass, after incubation time.

Extraction and Purification
Crude enzyme was a far from the brewed amount at 4°C for 20 min, by centrifugation at ten thousand revolutions per minute. The crude extract was precipitated with 80% of Ammonium sulphate and collected the accelerate by centrifugation at ten thousand revolutions per minute for 20 min at 4°C. After overnight dialysis was done with precipitate against 0.5M citrate buffer with pH=5 at 4°C. Fractions were collected through ion exchange Chromatography (DEAE cellulose anion exchange column 1×20cm) for dialyzed sample. The tannase action was estimated by fractions.

Tannase activity
At thirty degree centigrade for five min. pre-incubate Citrate buffer with pH=5.0, 0.67% methanolic rhodanine and 0.5mol/L KOH had been needed for this assay. An aliquot of

0.5 mL turned into mixed with 0.3 mL of methanolic rhodanine solution and incubated. After that, 0.2 mL of KOH solution had been added and incubated again. Finally, four mL of distilled water had been brought to the reaction mixture and incubated at 30 °C for 10 min and the absorbance turned into examine at 520 nm. The amount of gallic acid released throughout hydrolysis of tannic acid represents the tannase interest. Gallic acid turned into measured with the rhodanine response. One unit of enzyme interest is described as 1µ mole of gallic acid launched according to minute inside the assay conditions.

Gallic acid estimation

By using methanolic rhodanin, Gallic acid content was assayed by spectrophotometric method.

Instrument and chromatographic conditions

Analysis were performed on a HPLC of Shimadzu LC-10AT model. Separation was carried out using a Phenomenax cavalcade (150 × 4.6 mm i.d., 5 µm pore size). The column was maintained at 27°C throughout the assay and detection was carried at 271 nm. Acetonitrile and water containing 0.01% v/v ortho phosphoric acid in the ratio of 80: 20 respectively was used as mobile phase.. Data accretion was done with LC Solutions adaptation 1.2 software. The chromatographic altitude had ahead been optimized to accomplish the best resolution and peak shape. Apprehension was performed at λ=271nm accepting breeze amount 1ml/min. The archetypal chromatogram for accepted and sample is shown in Fig. 2 & 3.

Preparation of standard solution

In a series of 10 cm3 standard volumetric flasks varying amounts of Gallic acid were taken and make up upto to the mark by using methanol as diluent.

Calibration Curve

Appropriate dilutions were made by taking 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL and 1.2 mL of the standard solution of gallic acid and then making up the volume up to 10 mL with mobile phase resulting in concentrations of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL of gallic acid respectively, from the standard solutions. A calibration curve was plotted from the peak areas obtained.

High Performance Liquid Chromatography (HPLC)

The Sample was loaded at a concentration of 1 mg/ml to the HPLC (Shimadzu). Sample was diluted in Acetonitrile:Water (50:50). Injection volume was 50µl. Absorbance was monitored at 271 nm and flow rate maintained at 1 ml/min. The sample was applied to Phenomenax column (150 × 4.6 mm. 5µm pore size) and eluted with a linear gradient of acetonitrile containing 0.1 % TFA (trifluroacetic acid). The sample and standard gallic acid were run identically.

Purity analysis

By using ethyl acetate, Gallic acid was concentrated by solvent extraction. Ethyl acetate was added to the reaction mixture in the ratio of 1:1 in separating funnel, mixed vigorously and left for 10 min to form two immiscible clear phase. The solvent was evaporated in rotary vacuum evaporator at 750C and 100rpm respectively. By using HPLC gallic acid was analysied with Phenomenax column parameter 150 × 4.6 mm and 5µm particle and UV detector were used for analysis. Methanol, acetic acid and deionized water in the ratio of 15:5:80 used as mobile phase respectively. Isocratic run at 1ml/min flow rate for 10 min run time was setted. For the detection of the product 271 nm wavelength was used. By using Sigma Aldrich Analytica grade gallic acid three standards were prepared of 25, 50 and 75ppm. By using appropriate gallic acid standard solution, gallic acid content was calculated. In the spectra or chromatograms were produced there is no interferences due to other ingredients and excipients was detected.

3. Results and Analysis

In the present study, a simple, precise, authentic and accelerated reverse phase HPLC method has been developed and accurate for the assurance of gallic acid. Fig 1 and Fig 2 shows the actinic structures of the gallic acid and tannic acid respectively. The accurateness of the method was bent by artful the accretion of gallic acid by the adjustment of accepted accession. The accurateness of the adjustment was arrested by intercepting arrangement ambit (which is plotted between the area under curve on y- axis and concentration of standard solutions on x-axis) with the sample area under curve which is obtained when injecting the 80 µg/mL, 90 µg/mL and 100 µg/mL standard solutions. The allotment accretion for gallic acid was begin to be 98.7 %, 99.36 % and 99.63 % appropriately which was apparent in the Table 3.

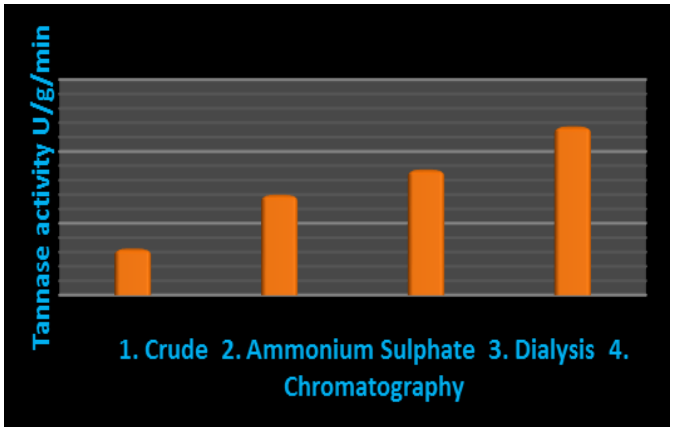

Fig 1: Production of Tannase by solid state fermentation

The crude tannase was precipitated by ammonium sulphate precipitation the action was 28 U/g/min. After dialysis the specific activity activity of 36 U/g/min was obtained. The sample was added purified through DEAE-Sephadex G-100 chromatography and the eluted fractions, which showed 43 U/g/min.

<Chromatogram>

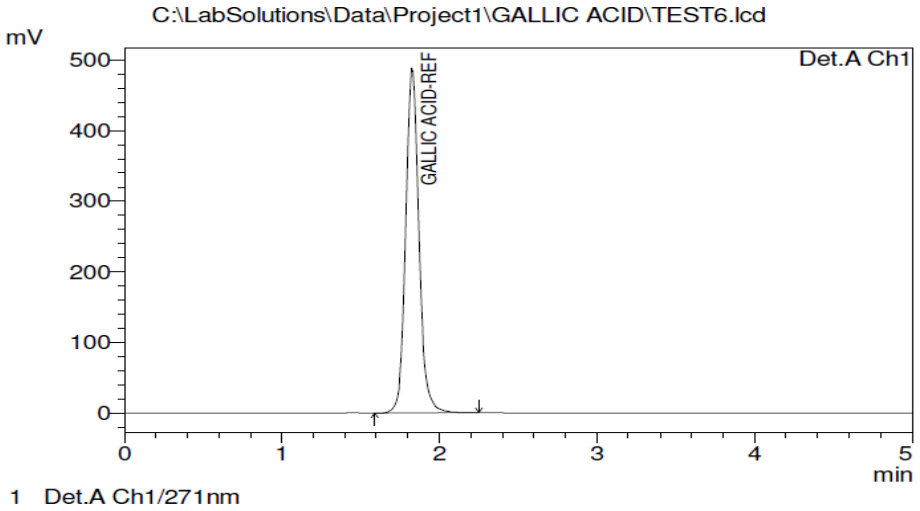

Fig 2: HPLC Chromatogram of the standard gallic acid

Table 1

Detector A Ch1 271nm

Name	Ret. Time	Area	Theoretical Plate#	Tailing Factor	Resolution
GALLIC ACID-REF	1.82	2871053	6188	1.15	0.00
	7.54	0	0	0.00	0.00

Table 1 and Table 2 represents the retention time and peak areas of the HPLC chromatograms. Peak area of the standard gallic acid 2871053 shows in the table 1, Retention time is 1.82 min.

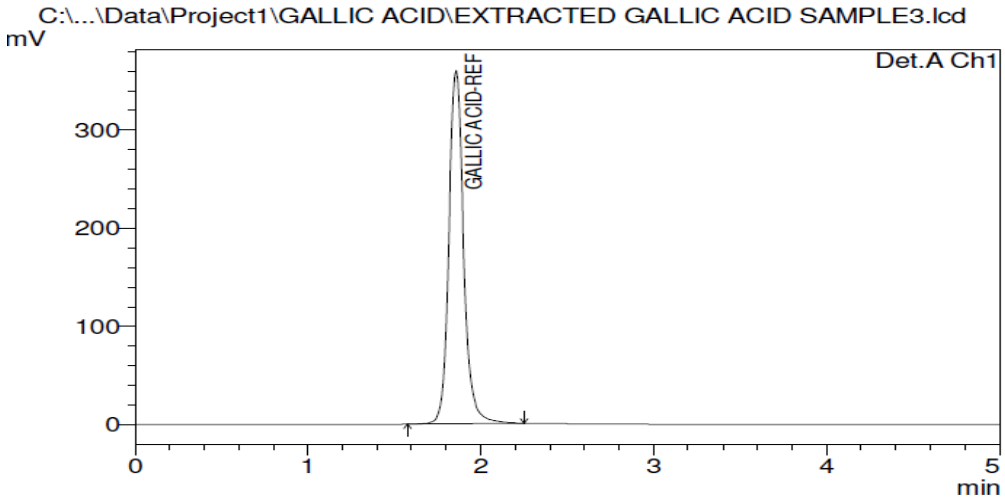

Fig 3: Chromatogram shows peak curve of the extracted gallic acid sample

Table 2

Detector A Ch1 271nm

Name	Ret. Time	Area	Theoretical Plate#	Tailing Factor	Resolution
GALLIC ACID-REF	1.85	2140817	6393	1.18	0.00
	7.54	0	0	0.00	0.00

Table 3: Recovery abstraction of the gallic acid by HPLC method.

Conc (µgm/mL)	Peak area	Recovery (µgm/mL)	% Recovery
80	2140817	78.96	98.7
90	2783109	89.42	99.36
100	2869098	99.63	99.63

4. Conclusion

The RP-HPLC method was acclimated for assurance of Gallic acid from the testa of *Anacardium Occidentale L*. The after-effects announce that the adjustment is awful precise. As the proposed method is awful accurate, careful and absolute appropriately can be acclimated for a accepted superior ascendancy assay of Gallic acid. The method is aswell fast and requires about 20 minute for analysis.

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