JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/36080

SHORT REVIEW

A Review on Extraction of Tannins and Quantitative Determination of Ellagic Acid Using Different Analytical Methods

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Abstract

Tannins, integral to plant survival, serve diverse functions from herbivore defence to influencing plant development. Classified into hydrolyzable and condensed types, these water-soluble polyphenols contribute to the nutritional profile of plant-based meals, offering taste, colour, and potential health benefits. Extraction methods, including solvent and ultrasonic-assisted techniques, play a crucial role in obtaining concentrated tannins. Ellagic acid, abundant in plants like strawberries and grapes, garners attention for its health-promoting properties. Analytical methods such as spectrophotometry and chromatography, including HPLC, enable the precise identification and quantification of ellagic acid. These tools contribute to a deeper understanding of plant chemistry and its potential health implications. In a nutshell, tannins go beyond herbivore defence, influencing plant biology and human health. This review highlights their diverse roles, extraction methods, and the significance of ellagic acid, providing insights into the intricate world of plant polyphenols.

Keywords: Analytical Method, Ellagic Acid, Extraction, Tannins

1. Introduction

In 1796, Seguin coined the term "tannin" by adopting the old Celtic word for oak, referring to a plant extract's capacity to transform hide or skin into leather. Tannins (TNs), identified as secondary phenolic components of plants, are commonly sourced from plants and represent sustainable natural resources. Biogenetic processes give rise to TNs, originating as polymeric and oligomeric proanthocyanidins or galloyl esters or in the plant's secondary metabolism. Proanthocyanidins result from the phenylpropanoid and polyketide (malonyl-CoA) metabolic pathways, while gallic acids are directly formed from shikimate[1-6]. TNs, constituting the primary polyphenolic secondary metabolites, are predominantly found in leaves, roots, bark, stems, seeds and buds comprising 5–10 % of dehydrated vascular plant materials[7-9].

Tannic acid, also known as TNs, is an aqua-soluble polyphenol existing in various plant-based diets. Research findings indicate that the presence of TNs can have adverse actions on feed efficiency, net metabolizable energy, feed intake, growth rate, and protein digestibility in investigational subjects. Diets rich in TNs are generally perceived as detrimental to health. Nevertheless, recent research suggests that the main influence of TNs might be attributed to the body's less efficiently converting ingested nutrients into new molecules rather than solely suppressing food intake or digestion. Consuming foodstuffs comprising elevated levels of TNs, like herbal teas and betel nuts, has been linked to an increased risk of various malignancies, including oesophageal cancer. However, several studies have indicated that the carcinogenic activity associated with tannin (TN)-rich foods may be attributed to other components present in these foods rather than the TNs

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Article Received on: 04.01.2024 Revised on: 20.02.2024 Accepted on: 30.03.2024

themselves. Interestingly, numerous research findings have established a negative connotation between tea intake and cancer risk. This has been attributed to the hypothesized anticarcinogenic properties of the polyphenols and TNs present in tea. Studies suggest that certain TN compounds may have the ability to inhibit the mutagenic effects of some mutagens. Oxygen-free radicals generated by numerous malignancies and/or mutagens engage with biological macromolecules. The antioxidative characteristics of TNs, possibly associated with their anticarcinogenic and antimutagenic capabilities, provide an essential function in preventing oxidative damage to cells, particularly lipid peroxidation. Studies have revealed that TNs and related compounds reduce the production of superoxide radicals. Additionally, research has shown the antimicrobial attributes of TNs, as they impede the growth of various microorganisms, including bacteria, viruses, yeasts, and fungi. Furthermore, our investigation revealed that tannic acid and propyl gallate effectively inhibited bacteria associated with off-flavours in food, water, and produce, while gallic acid did not exhibit the same inhibitory action. Ripe fruits were thought to have antibacterial qualities due to the ester linkages that exist among gallic acid and polyols, which break down dissolved chemicals. Consequently, the TNs present in these fruits act as a protective barrier against bacterial and fungal infections. The food industry can leverage the antimicrobial properties of tannic acid to prolong the shelf life of products like catfish fillets. Additionally, studies have shown that TNs can expedite blood coagulation, lower blood pressure, decrease serum cholesterol, induce liver necrosis, and regulate immunological responses. The specific type and amount of TNs employed play a decisive function in determining these effects[10].

2. Classification of TNs

Two distinct types of TNs exist: hydrolyzable and nonhydrolyzable TNs, also termed condensed TNs. Hydrolyzable TNs consist of a polyhydric alcohol, like glucose, with hydroxyl groups that are incompletely or completely esterified by hexahydroxydiphenic acid (resulting in ellagitannins) or gallic acid (forming gallotannins). When gallotannins undergo hydrolysis through acids, bases, or specific enzymes, gallic acids and glucose are released. In ellagitannins, the lactonization of hexahydroxydiphenic acid produces Ellagic Acid (EA)[11].

Prominent gallotannins encompass Turkish TN, Tara TN, Chinese TN (tannic acid), Acer TN and Hamamelis TN. The Chinese gallotannin, derived out of nutgall, consists of nine to ten gallic acid molecules ester-bound to a glucose molecule. Turkish TN originates from *Quercus infectora*, Tara TN is sourced from *Caesalpinia spinosa*, Acer TN is obtained from Korean maple (*Acer ginnala*) leaves, and Hamamelis TN (*Hamamelis virginiana*) is extracted from hazelnuts. The simplest form of ellagitannin, corilagin, exists in plants such as *Terminalia chebula*, *caesalpinia* and *coriaria,* various species of schinopsis, and *Eucalyptus sieberiana*. Chebulinic acid and chebulagic acid, along with EA, are noteworthy ellagitannins. Brevilagins, another chemical component, can be extracted from *Casesalpinia brevifolia*. Dehydrigallic acid is present in sweet chestnuts. Agarobilla TNs can be transformed into carboxylic acid brevifolin. TNs that can undergo hydrolysis exist in the pods, bark, wood, fruits, leaves, and galls of herbs from various families, including Anacardiaceae, Fabaceae, Combretaceae, and Leguminosae[12]. The chemical constructions of all significant TNs are illustrated in Figure 1[13,14].

The structure of condensed TNs is more intricate compared to hydrolyzable TNs, and the complete details of their structures remain unknown. These polymers are primarily derived from flavan-3-ols, flavan-3,4-diols, or an amalgamation of both. While commonly referred to as condensed TNs, these polymers are accurately known as flavolans. Concentrated TNs are present in fruits, vegetables, red wine, cocoa, forage, and herbs, and are also found in legumes, sorghum, and finger millets.

Catechins, synonymous with flavan-3-ols, comprise four isomers of catechin molecules due to the existence of two asymmetric carbons at positions C-2 and C-3. These (+) and (-) catechins incorporate trans 2-phenyl and 3-hydroxy groups. The separation of (–)-epicatechin that exists in cacao beans is achievable. In green tea, the primary phenolic substances are (–)-epigallocatechin and its 3-gallate, accompanied by additional phenolic components like (+)-gallocatechin, (+)-catechin, and (–)-epicatechin and its 3-gallate. Flavan-3,4-diols are classified as leucoanthocyanins

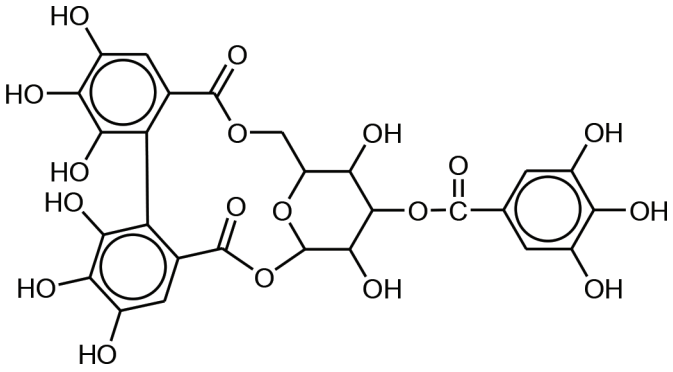
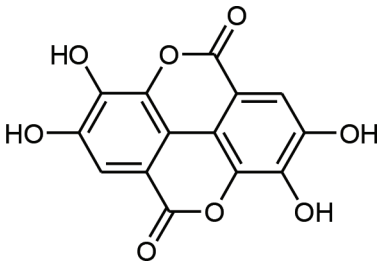
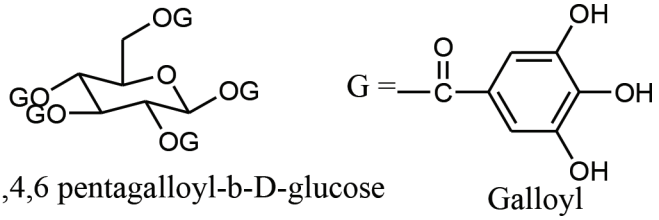
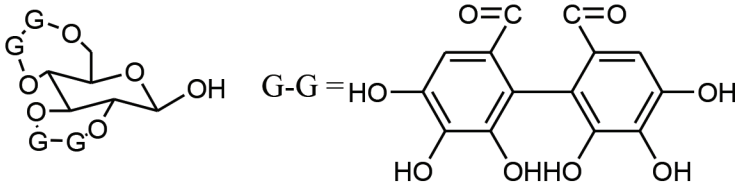
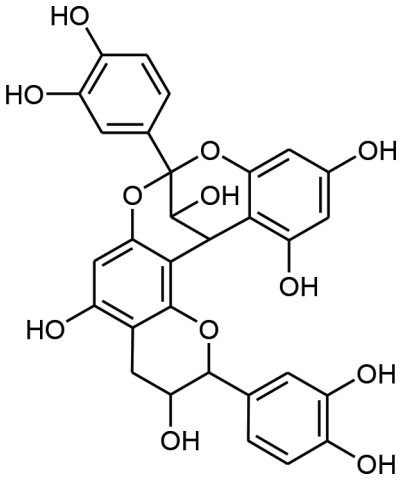
Corilagin	
Ellagic Acid	
Gallotannins	 1,2,3,4,6 pentagalloyl-b-D-glucose Galloyl
Ellagitannins	 2,3-4,6-di-HHDP-β-D-glucose
Proanthocyanidins Dimer type A	

Figure 1. Chemical constructions of significant TNs.

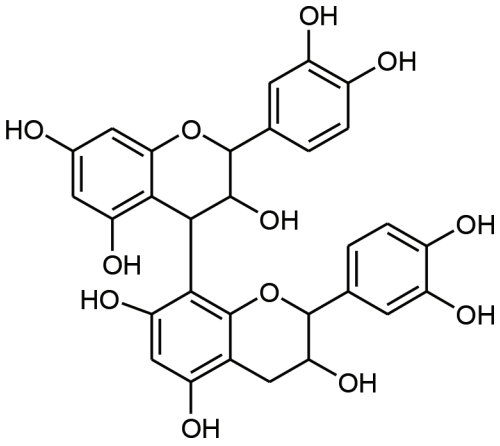
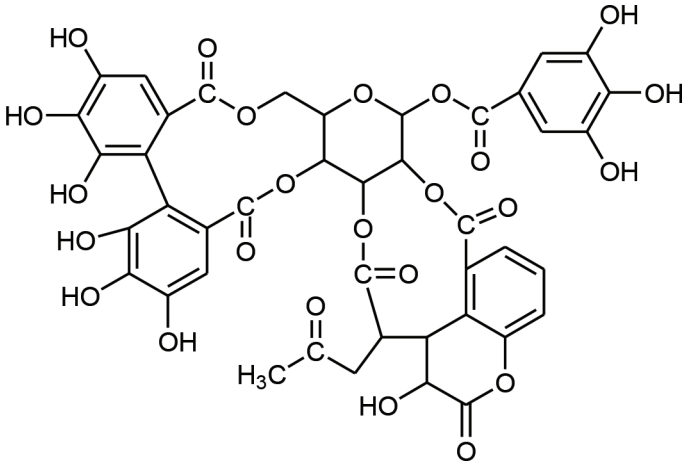
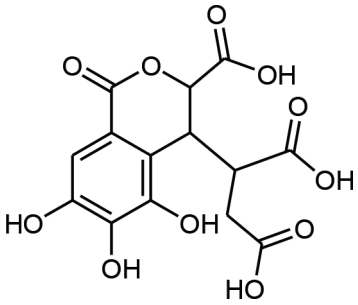
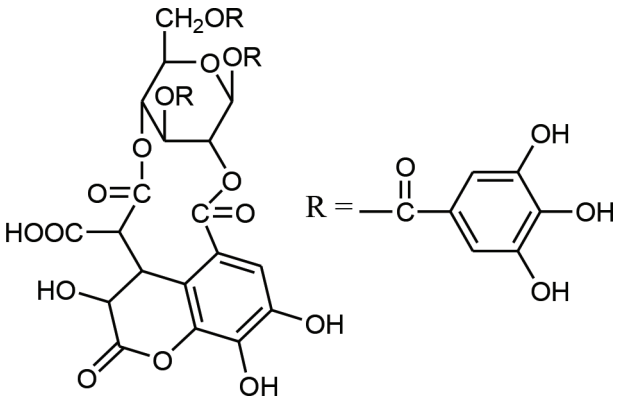
Proanthocyanidins Dimer type B	
Chebulagic Acid	
Chebulic Acid	
Chebulinic Acid	

Figure 1. Continued.

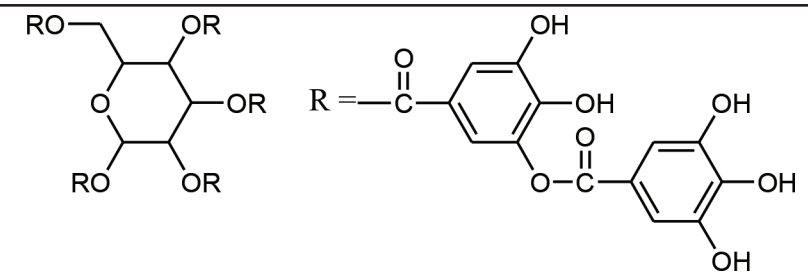
Chinese TNs	

Figure 1. Continued.

because they get polymerized into phlobaphene-like substances and generate anthocyanidins when subjected to heating in an acidic solution. A flavan 3,4-diol fragment comprises eight isomers due to the presence of asymmetric carbons at positions C-2, C-3, and C-4. Among the flavan-3,4-diols with identified structures are guibourtacacidin, leucocyanidin, leucopelargonidin, (–)-teracacidin, leucodelphinidin, (–)-melacacidin, (+)-leucorobinetinidin.

Proanthocyanidins, existing in the form of polymers, trimers or dimers are fundamentally composed of epicatechin and catechin. Complex TNs, on the other hand, represent a combination of catechin, gallotannins, ellagitannins and various other small polyphenols[15-19].

3. Sources of TNs

TNs have been traced in a wide variety of edible plants commonly used as animal feed and human food. Food sources include winged beans, dry beans, sorghum, carobs, millets, barley, faba beans, peas, pigeon peas, and various legumes. Additionally, numerous fruits like strawberries, grapes, plums, apples, blackberries, dates, persimmons, hawthorns, peaches, pears, raspberries, bananas and cranberries also contain substantial levels of TNs[20-26]. Phenolic substances exist in wines and teas[24,27]. TNs have also been identified in several forages, including sainfoin, trefoil, lotus, lespedeza and crownvetch[28,29].

4. Extraction of TNs

TNs are a significant area of research owing to their extensive potential applications and widespread natural occurrence. Nevertheless, several factors, such as biological origin, tree age, geographical location, species and the wood's position (whether in the inner or outer layers of the tree), can influence the quantity of this compound in a plant[30,31]. Extensive research has been prompted by the numerous potential applications of TNs. However, the diverse nature of TNs poses a significant challenge to their commercialization, particularly in the extraction process. Extraction methods for TNs are not consistently standardized and can exhibit considerable variations. Contaminants, including minerals, stilbenes, and sugars, may be introduced into the extracted TN through byproducts generated during the extraction process[32,33]. The presence of carbohydrates in TNs, for instance, diminishes their antifungal effects[34,35], and complications arise when TNs are impregnated into wood for preservation[34,36].

The ultimate impurity level is impacted by various aspects, for instance, type of solvent, pressure, particle size, temperature, duration and the ratio of solid to solvent. Two specific factors identified as influencing the number of carbohydrates in the extracted TNs are temperature and the solid-to-solvent ratio[32,35,37]. While TN extraction is an essential process, precise operational parameters are crucial. TNs are commonly extracted from various plant materials (bark, wood, stem, leaf) using either hot water or water in combination with different solvents. Researchers have employed a range of solvents, including acetone[38-44], methanol[45], ethanol[46,47], sodium hydroxide[48-50], diisopropyl ether[32,35], ethyl acetate[40], sodium sulfite[42] using or not using water.

Dentinho *et al.,*[39] eliminated TNs from desiccated leaves and green shoots of rockrose (*Cistus ladanifer*) using acetone, while TNs from pine (*Pinus radiata*) barks[45,51] were extracted using a solution of 75% methanol with water. In other investigations, TN extraction from fever tree (*Acacia xanthophloea*)[46,47,52]

and marsh rosemary (*Limonium delicatulum*)[53] utilized a mixture of 50–80% methanol and water through infusion and maceration. Microwave technology was employed in extracting TN from black wattle (*Acacia mollissima*) barks. Furthermore, methanol (60%) and ultrasonic technology were applied to extract TNs from acorns[54].

Based on the research findings[45,52], the efficiency of methanol extraction seems to outstrip that of water extraction. TNs from Mussoorie berry (*Coriaria nepalensis*) were extracted using NaOH, with an ideal concentration of 0.22%[55]. While N-hexane can be used for TN extraction, this method is not widely employed due to its extremely low yield. The addition of additives, such as acids and enzymes, to the solvent can enhance both TN quality and production[56]. In a study, it was observed that the extent of total TN in the solution increased when the amount of enzyme in the solvent was elevated[57,58]. The researchers utilized pectin-lyase and polygalacturonase in conjunction with grape skin for the extraction of TN. Enzymes were employed to break down the cell wall and create openings[58-60], enhancing the discharge of TNs from grape skin. Similarly, the addition of 0.01% and 70% ascorbic acid and acetone respectively facilitated phloro, whole and condensed TNs extraction from microalgae[61]. While there are various methods for TN extraction, the hot water extraction technique remains the most prevalent and widely utilized in both academic and commercial contexts[62,63] and academic research[64]. The widespread popularity of the hot water extraction method can be attributed to its cost-effectiveness and simplicity. According to certain studies, the highest concentration of both condensed and hydrolyzable TNs was achieved when hot water served as the solvent[65]. This outcome may be influenced by factors such as the plant type, raw materials employed, temperature, duration of extraction, and particle size. Several other parameters, such as the extraction temperature and solvent used, can affect the final yield. However, the specific temperature varies based on the materials, extraction timeframes, techniques, and particle sizes.

The TN extraction method detailed by Bello *et al.,*[37] involved using hot water to extract TNs from the bark of spruce (*Picea abies*) trees at a temperature of 85°C, while pine (*Pinus radiata*) trees were extracted at 90°C. The type of diluent determines the maximum temperature, which can reach 120°C. The TN extraction from umbu (*Spondias tuberosa*), red angico (*Anadenanthera macrocarpa*), and jabuticaba (*Myrciaria jabuticaba*) barks used water, and a temperature of 105°C was required[66]. On the other hand, 75°C was shown to be the sweet spot for TN extraction from manjakani galls (*Quercus infectoria*)[67]. It was observed that a lower temperature was required when using water as the extraction solvent[53]. In cases where water was employed as a solvent alongside methanol or ethanol, the temperature ranged from 60 to 120°C. However, better outcomes were achieved at higher temperatures[46,52]. For TN extraction from bark using NaOH as the solvent, a temperature of 82°C was determined to be optimal[47,48]. In instances where acetone was employed as an extracting liquid for TN, the processing temperature was either at room temperature or set at 60°C. Apart from employing advanced technologies such as ultrasound and microwave technology, which allow for lower processing temperatures, the application of pressure also contributes to TN extraction at reduced temperatures. Nevertheless, it is imperative to keep in mind that the particle size plays a significant role in influencing the extraction process[68-73].

For optimal outcomes, it is advisable to finely grind[48,50] these botanical elements to sizes of 0.5 mm and 0.55 mm before initiating any extraction procedure. In the extraction of TNs from barks and wood chips, factors such as efficiency, duration, temperature, solvent type, raw material, and method are considered. It's worth noting that these factors impact particle size in distinct ways. Smaller particle sizes facilitate quicker TN extraction and also enhance the accessibility of solvents to the particles[45,51,54].

Beyond the previously outlined TN extraction methods, several advanced techniques are available, including pressurized hot water extraction, infrared-assisted extraction, ionic liquid-assisted microwave extraction, and supercritical approaches. The pressurized water extraction method requires a pressure of 22.1 MPa and a temperature of 374°C[50,74]. The use of ionic liquids has been made as a prior treatment in advance of microwave extraction, and these liquids encompass various compounds such as 1-butyl 3-methylimidazolium OH, 1-ethyl-3-methylimidazolium Br, 1-decyl-3-methylimidazolium Br ,1-hexyl-3-methylimidazolium Br, 1-octyl-3-methylimidazolium Br, 1-butyl

3-methylimidazolium BF4, 1-butyl 3-methylimidazolium Cl, 1 butyl 3-methylimidazolium NO3, and 1-butyl-3-methylimidazolium Br[70]. In the process of extraction using the infrared method, an infrared lamp is utilized. The effectiveness of this procedure is influenced by factors such as the infrared heater wavelength, the remoteness between the material and the infrared source, and the choice of solvent[71-73]. Additionally, investigations have been conducted on TN extraction through gamma radiation. Certain solvents are essential to immerse the material in this process. However, it is important to note that gamma radiation can also irradiate both materials and solvents[74]. An advanced approach for TN extraction involves the use of supercritical technology, employing solvents like butane, nitrous oxide, pentane, carbon dioxide, fluorinated hydrocarbons and sulfur hexafluoride. The selectivity of CO_2 can be enhanced by introducing a co-solvent or modifier[75-78].

The duration of the extraction process has a significant role in estimating both the purity of TNs and the capability of extraction, as evidenced by studies[54,65]. In essence, a prolonged extraction period leads to higher TN content. However, it's essential to note that an extended extraction time may cause gradual damage to the plant cell structure within the solvents, potentially compromising the quality of the extracted TNs[61]. Another critical factor influencing the TN extraction mechanism is the solvent-to-solid ratio, as indicated by relevant research[39,55]. Enhanced mixing is attainable when the solvent-to-solid ratio is increased. Consistent blending of solvents with the particles facilitates the extraction process. It's important to note, however, that this ratio exclusively impacts the particles and does not have any influence on the solvent, which is the controlling factor in TN extraction.

5. Quantitative Determination of EA

5.1 Spectrophotometry

UV Spectrophotometry is a technique utilized to quantity the light absorption of a chemical substance by observing the light intensity that travels through it. Researchers from various disciplines commonly utilize this approach as a fundamental and preliminary stage in their investigations. This data offers a comprehensive view of the chemical's solubility and absorption characteristics.

EA exhibits maximum absorption with log ε values of 3.93 and 4.60 in ethanol at 366 and 255 nm, respectively, as reported by PubChem in 2019. Due to its intricate structure and properties, Chen Jia-hong and colleagues attempted to solubilize EA in a diluted solution of NaOH, as it proved insoluble in water and various organic solvents. They recorded the UV distinctive absorption peak at 357 nm for EA[79]. According to Budavari and colleagues, the λmax for EA is at 357 nm. Furthermore, they noted that EA is insoluble in water[80] and exhibits only occasional solubility in alcohol. Bala and co-researchers, employing a validated UV spectroscopy approach, explored the methanol and water solubility of EA. They stated the greatest absorbance at 255 nm and 360 nm[81]. The EA's peak absorptions were identified at 255 nm, 254 nm, and 360-368 nm in numerous studies[82,83].

EA is a slightly acidic substance, and its solubility rises with an increase in pH. The reported pKa value for EA is 5.6. Given its four weakly acidic phenolic groups, it is expected to exhibit four dissociation constants. At pH levels lower than 5.6, EA is present in a monodeprotonated state. However, deprotonation takes place at the two hydroxyl group locations above pH 5.6[84-86]. The results also indicated that EA enhances its solubility by shedding two hydrogen atoms per molecule. When efforts were made to elevate the pH to enhance the solubility of EA, a dark greenish-brown colour emerged, potentially because of the hydrolysis of lactones at pH 9.6 using 5-10 M NaOH[81].

EA displays multiple absorption regions in infrared spectroscopy. Goriparti and collaborators, after scanning in the range of 4000 to 10,000 cm^{-1}, performed an analysis of the IR spectrum for total polyphenols. Their findings revealed a broad band stretching for -OH in the array of 2800-3700 cm^{-1}, along with a C=O stretching at 1725 cm^{-1}. Vibrational bands indicative of an aromatic ring were observed in the range of 1669 to 1500 cm^{-1}. Signals at 1190 and 1052 cm^{-1} were identified as arising from ester bonding. Additionally, another signal at 751 cm^{-1} was associated with the bending vibration of aromatic C-H[87].

5.2 Chromatography

For qualitative and quantitative assessment of EA in the formulations and extracts, multiple chromatographic methods have been reported. A

more advanced and mechanized kind of thin-layer chromatography is HPTLC. It boasts enhanced capabilities in detection and separation. The primary uses of this method include phytochemical investigation, quantification of phytoconstituents, formulation fingerprinting, and quality verification of formulations[88].

5.2.1 High-Performance Thin Layer Chromatography (HPTLC)

Dalavi and team examined the impact of EA on antibacterial action by utilizing seed extract from *Syzygium cumini*. Throughout a six-month investigation, they employed HPTLC to assess the effects of accelerated storage on plant markers and the extract's antibacterial properties. EA peaked at an Rf of 0.47 ± 0.02. The outcomes indicated a decrease in the antibacterial efficacy of the extract and a corresponding reduction in the % assay over the half-year trial period[89].

HPTLC was employed to quantify the EA concentration in the *Abrus precatorius* Linn, *Phyllanthus maderaspatensis* Linn seeds, and the florets of *Nymphaea alba* Linn. Extracts were made using the Soxhlet extraction method, and the results were diluted with methanol. The method precision (%RSD) for gallic and EA was observed to be 0.083 and 0.78, correspondingly, according to the research. Repeatability values were reported as 1.07 and 1.50 (% CV). Furthermore, they conducted a recovery analysis at two dissimilar levels to assess the accuracy of the simultaneous technique. The average percentage recovery for gallic and EA in the entire plant was reported as 101.02% and 102.42%, respectively[90].

EA was identified in the *Woodfordia fruticosa* plant obtained from Karnataka, Himachal Pradesh and Telangana. To prepare the extract, the sample underwent reflux with 70% ethanol (50 ml) for 30 minutes. The results indicated that the extracts containing EA exhibited bands at UV 254 and 366 nm, with an Rf value of 0.30, consistent with the standard EA[91].

Bazylko and their co-researcher conducted fingerprinting examinations on eleven kinds of Potentilla L. (Rosaceae) gathered at different times and extraction was performed using methanol. This research concentrated on HPTLC fingerprinting techniques for various polyphenolic substances, with a specific focus on EA in Potentilla species. The method utilized HPTLC silica gel 60 F254 as the stationary phase, and a solvent mixture made up of toluene, ethyl formate, and formic acid (6:4:1 v/v/v). They stated the occurrence of EA at Rf 0.13 for the examined species[92].

HPTLC was employed to assess the polyphenolic constituents in diverse plant fragments of Rosa hybrid. Across the leaves, early buds, wood, stems, buds before flowering and florets of the Rosa hybrid plant, a total of sixty chemicals, predominantly EA, were identified[93]. The information concerning the parameters of each HPTLC method is provided in Table 1[88].

Table 1. Quantitative assessment of EA by HPTLC methods

Sample	Matrix	Extraction component	Chromatographic conditions	Detection wavelength	RT (in Min)	References
HPTLC Assessment of Extract						
Syzygium cumini seed	Extraction	Ethanol	Toluene: ethyl acetate: formic acid (6:6:1.5 v/v/v)	271 nm	0.47 ± 0.02	89
Abrus precatorius, Nymphaea alba Linn, *Phyllanthus maderaspatensis*	Extraction	Methanol	Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2 v/v/v/v)	280 nm	0.46	90
Woodfordia fruticosa	Reflux	HPLC water	Toluene: chloroform: ethyl acetate: formic acid (2:6:6:2 v/v/v/v)	254 & 366 nm	--	91
Potentilla species	Extraction	Methanol	Toluene: ethyl formate: formic acid (6:4:1 v/v/v)	254 nm	--	92
Rosa hybrida	Extraction	--	ACN:H_2O:HCOOH (50:50:5 v/v/v)	270 nm	--	93

5.2.2 High-Performance Liquid Chromatography (HPLC)

It is an advanced approach utilized for separating constituents from diverse specimens. The separation process hinges on the type, molecular mass, and chemical structure of the sample. HPLC enables the investigation of phytocomponents, their degradation products, and potential derivatives. This method is capable of assessing analytes even at very small amounts, especially in the occurrence of co-eluting constituents. Presently, HPLC proposes an additional benefit for exploring biological matrices and polyphenolic plant-derived substances. This includes the capability to connect two or more columns in a swapping manner, along with a wide array of commercially accessible columns featuring appropriate properties and next-generation sorbents. With these distinctive features, HPLC is acknowledged as the popular industry-standard technique for analyzing EA[94].

To quantify and identify EA present in extracts derived from *Terminalia catappa* bark and fruits, *Terminalia chebula*, *Terminalia arjuna* and *Terminalia bellirica*, a rapid HPLC-PDA methodology was developed which is also validated. The authors specify that the limits of measurement and detection for EA are 1.0 mg/mL and 0.5 mg/mL, respectively. This methodology is appropriate for both quantification and quality assurance of extracts and herbal preparations obtained from all Terminalia species[95].

An HPLC methodology was employed to estimate the quantity of EA in an extract of *Epilobium angustifolium*, commonly known as the Canadian willow plant. The authors observed an RSD of less than 2.0 % for the peak area of the standard solution and a correlation coefficient (r2) exceeding 0.999. The accuracy of the technique was evaluated through the mean percentage recovery, which fell between of 98–102 % which is acceptable[96].

The estimation of EA from *Eugenia uniflora* L. (Myrtaceae) leaf extracts in ethanol was performed utilizing the HPLC-UV technique, which was subsequently optimized and validated. Employing ultrasound-assisted extraction (UAE) with a Box Behnken design and response surface approach, the research investigated the influence of temperature, extraction duration, and ethanol concentration on EA. As reported by Assuncao and collaborators, the highest recovery rate of EA was achieved using UAE with ethanol as the solvent[97].

RP-HPLC was employed to explore the bioactive component in *Geum rivale*. Owczarek and collaborators examined a total of eleven phenolic acids in the plant's aboveground parts and eight in its underground sections. The extract was prepared using a Soxhlet apparatus with petroleum ether and chloroform. Furthermore, they observed that EA had a retention time of approximately 40 minutes[98].

Five different plants, namely the husk of Punica granatum, the leaves of *Turnera diffusa*, the branches of *Euphorbia antisyphyllitica*, the leaves of *Jatropha dioica*, and *Flourensia cernua*, were gathered, finely crushed, and extraction was performed together with HPLC study. The results indicate that dissimilar plants, including *Turnera diffusa* (0.87 ± 0.59), *Punica granatum* turning (33.79 ± 7.43), *Euphorbia antisyphyllitica* (2.18 ± 0.39), *Flourensia cernua* (1.59 ± 0.96), *Jatropha dioica* (0.81 ± 0.43) and *Punica granatum* red (12.80 ± 5.83) mg/g, contain varying concentrations of EA[99].

Using a Betasil C_{18} column and two distinct solvent systems, one comprising of acetic acid: water (2:98 v/v) and the other of acetic acid: acetonitrile: water (2:50:48 v/v/v)—Aaby and colleagues performed an HPLC study on *Fragaria x ananassa*. As per their findings, EA exhibited a retention time of 55.1 minutes at 368 nm[83].

The preliminary detection of polar substances in six dissimilar organs (early buds, wood, shoots, buds before blooming flowers and leaves) of the Rosa hybrid plant involved HPLC-DAD analysis. In their study, Riffault and team observed that EA had a retention time of 29.2 minutes at a wavelength of detection at 270 nm[93].

The retention time was 10.6 minutes for EA in the leaves of *Myricaria bracteata*. The mobile phase comprised a blend of methanol and 0.1% aqueous orthophosphoric acid, with a ratio of 50:50 (v/v)[100].

EA was identified in both processed and acidified methanolic extracts through the analysis of grape seed extracts using the HPLC-DAD system. The grape seed extract was determined to have 0.08 mg/g of EA, with a retention time of 22.7 minutes and a high regression coefficient (R^2) of 0.9975[101].

5.2.3 Gas Chromatography-Mass Spectrometry (GC-MS)

To identify, characterize, and quantify volatile compounds, GC is an established and trustworthy analytical tool. Its effectiveness stems from its robust

separation capabilities and highly sensitive detection, making it a valuable tool in analytical processes. Despite its numerous benefits, the use of this methodology is restricted to essential oils due to the potential breakdown of compounds under heat. However, when coupled with mass spectrometry, this approach offers excellent selectivity and sensitivity[102,103].

Geum rivale, a member of a specific plant family and subfamily known for containing substantial levels of TNs, including polyphenolic components such as EA, underwent analysis using GC-MS for both its aerial and underground parts. The extraction process utilized the Soxhlet apparatus, with the extract made using petroleum ether and chloroform. The GC-MS investigation was done with an Agilent 6890N gas chromatograph and an Agilent 5973-MS detector, employing a 70 eV electron impact ionization potential. Owczarek and co-authors stated that the underground parts of the plant contain approximately 130 mg/1g of TNs[104].

TNs were examined using GC-MS in the wine, grape and oak extracts. According to the paper, GC-MS analysis exposed the occurrence of EA in wine extract and oak. Utilizing Agilent GC 6890N/MSD 5973B equipment, the analysis was carried out. EA was reported to have a retention duration of 33.84 minutes[105].

The phytocomponents of *Salacia chinensis* L. have undergone GC-MS analysis using a 60 M RTX 5MS nonpolar column. The analysis employed a split-less mode method for the injection of 2 µl samples. Mass spectra were saved within the 35-650 AMU range, utilizing an ionization energy of 70 eV. The statistical outcome was assessed using GraphPad Prism. Using a variety of extraction methods, the author determined that the root, fruit pulp, seeds, and stem all contained EA equal in terms of per gram of dry weight[106].

5.2.4 Liquid Chromatography – Mass Spectrometry (LC-MS)

Liquid chromatography employs a collection of analytical procedures, like UV, fluorescence, and electrical conductivity, to separate sample components based on dissimilarities in their retention ability or affinity for either the stationary or mobile phase. Subsequently, the identified constituents are detected according to their distinctive properties. Mass spectrometry (MS) employs extremely sensitive recognition methods that ionize sample constituents through numerous techniques like laser desorption, chemical ionization, fast atom bombardment, and electrospray ionization. The generated ions are subsequently separated in a vacuum based on their ratios of mass-to-charge, and the strength of the respective ions is quantified. Consequently, LC-MS systems conglomerate the exceptional qualitative potential of mass spectrometry with the remarkable separation and resolving capacity of liquid chromatography. The mass spectrometer generates and identifies charged ions, providing knowledge on the structure, molecular mass, uniqueness, and amount of particular sample constituents through the data obtained from LC-MS. By comparing the ethanolic extract of pomegranate peel to a reference standard, EA was identified by LC-MS analysis. According to the report, the existence of EA is established by the [M-H]- at m/z 301. Using the electrospray ionization approach, the identical constituent in MS-MS yields fragments at m/z 258, 229, and 185[107]. Four species of *Drosera* were used in the development of the method and measurement of EA derivatives: *D. intermedia*, *D. rotundifolia*, *Drosera anglica*, and *D. madagascariensis*. Several additional chemicals were found and identified by LC-MS in addition to EA. An UltiMate 3000 RSLC-series equipment attached to a 3D quadrupole ion-trap mass spectrometer outfitted with an orthogonal ESI source was used to perform the LC-MS study of Droserae herba. EA exhibited [M-H]– at 300.9, with prominent portions identified at 283.8, 270.8, 256.8, 228.9, 212.8, 200.8, and 184.9[108]. HPLC-ESI-QTOF-MS/MS was employed to identify EA and its derivatives in ethanolic extracts of *Phyllanthus emblica*, *P. niruri, P. fraternus* and *P. amarus*[109]. Lee and colleagues identified EA in Muscadine Grapes using HPLC-ESI-MS. They observed a base peak at m/z 301 in [M-H]-, and the MS2 spectra displayed peaks at m/z 301, 284, 257, 229, and 185 for EA[110]. *Fragaria x ananassa* was subjected to LC-MS analysis by Aaby and colleagues. They did not divide and allowed the LC elution to access the ESI interface directly. The dry temperature was kept at 350°C, the capillary voltage was kept at 3.5 kV, the pressure of the nebulizer was fixed at 40 psi, and the dry gas flow rate was 10 L/min. Common fragments of EA, such as a [M-H]- peak at m/z 301 and MS2 fragmentation ions

in negative mode at m/z 257, 229, and 185, have been detailed, including a peak at [M-H]- at m/z 301 and MS2 fragmentation ions in negative mode at m/z 257, 229, and 185[83]. Chernonosov and colleagues identified EA in the leaves of *Myricaria bracteata*. Their findings indicate that EA, represented by the molecular ion [M-H]- at m/z 301, also exhibits fragment ions at m/z 284, 257, 229, and 185[100].

5.3 Miscellaneous Methods

Another often-used technique to ascertain the amount of active ingredients from a plant extract sample is Capillary Zone Electrophoresis (CZE). This method is being developed by a broad group of scientists as a substitute instrument for identifying the acid. In Eucalyptus globulus wood, Costa and colleagues have created a technique to identify EA using CZE and have subsequently contrasted the findings with those obtained through GC-MS. Furthermore, stated that this marks the inaugural CZE application in industrial streams to detect EA during the production of cellulosic pulp. EA findings from the GC-MS were 1232 to 1083 mg/kg, while those from the CZE were 959 to 986 mg/kg. This demonstrated that the results for EA from the two approaches are not substantially different[111].

Quantitative analysis of pomegranate rinds was performed by Capillary Electrophoresis (CE) by Zhou and colleagues, and the outcomes were compared with HPLC. The limits of detection for CE and HPLC were 2.2 and 2.8 µg/mL, correspondingly. The usual retrievals for CE were 96.52 ± 2.8%, and for HPLC they were 98.32 ± 1.2%. The published findings show that while the HPLC method produced higher precision, the CE method needed less solvent and produced enhanced column efficiency[112].

6. Conclusion

TNs represent significant naturally occurring compounds found in various forms, particularly in plants. They exist primarily as condensed and hydrolyzable TNs, predominantly in barks, leaves, fruits, fruit shells, stems, seeds, and shoots. TNs can be extracted from these sources utilizing only water or in combination with numerous solvents like ethanol, sodium hydroxide, acetone, methanol, and ionic liquids. Innovative approaches like microwaves and ultrasonication have proven effective in extracting TNs. Given the reliance on technology and solvents for the utilization of TNs, ensuring their purity is essential. Enhanced TN quality can be achieved by regulating factors such as temperature, solid-to-solvent proportion, particle size, origin, and time taken for extraction. EA is a significant plant compound classified as a type of TN and holds importance as a phytoconstituent. Given the reputation of EA in addressing numerous illnesses, it is crucial to explore diverse quantitative methods for detecting and quantifying EA sourced from the environment. The findings from all the methodologies discussed in this investigation encompass a series of experiments carried out on a variety of samples and instruments by different researchers to determine the EA levels. As per the outcomes, these techniques apply to both raw materials and finished products, aiding in the assessment of EA concentration-a pivotal factor in the stability of formulations and products.

6. Acknowledgement

We would like to express our heartfelt thanks to those who assisted in preparing this manuscript. Additionally, we would like to convey our thanks for the continuous support and help provided by Sumandeep Vidyapeeth Deemed to be University, located in Piparia, Vadodara.

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