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RESEARCH ARTICLE

Derivatization of Gallic Acid with amino acids for accentuation of its antioxidant potential

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ABSTRACT

Phenolics like gallic acid have been known for their anti oxidant activity since long time. Many attempts for increasing antioxidant potency of phenolics have been made utilizing synthetic and drug discovery tools. In an attempt to improve the antioxidant potential of gallic acid, it was decided to introduce a few amino acids into the structure of gallic acid and evaluate their antioxidant potential in vitro. The amino acid derivatized gallic acid molecules were confirmed for their formation by spectral studies and the in vitro antioxidant potential was evaluated using DPPH free radical scavenging protocol and hydrogen peroxide free radical scavenging assay method. The results revealed that presence of cysteine residues in the amino acids were particularly beneficial for the antioxidant potential.

Keywords: Gallic acid, antioxidant, amino acids, derivatization, radical scavenging

Introduction

Medicinal plants have been used in all cultures as sources of medicines (Plotkin, 1991) as therapeutic agents since the origin of human civilization through the middle Paleolithic age around 60,000 years ago (Solecki, 1977). The ancient texts of India and China mention exhaustive depictions about the use of a variety of plant-derived medications (Ahmad et al., 2006). An impressive number of modern drugs have been isolated from natural sources. According to the World Health Organization (WHO), it is estimated that about 80% of the population in Asia, Africa and Latin America rely on traditional medicine (Hack- Seang, 2005). Of the 252 medicines considered as basic and essential by the WHO, 11% originate exclusively from plants and a considerable number is synthesized from naturally occurring precursors. Digitoxin (from Digitalis spp.), vincristine and vinblastine (from Catharanthus roseus), quinine (from Cinchona spp.), atropine (from Atropa belladona) and morphine and codeine (from Papaver somniferum) are some examples of important plant-derived drugs. The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them. The most important of these constituents are the alkaloids, glycosides, tannins, flavonoids and polyphenolic compounds. The most abundant and the most widely studied bioactive compounds obtained from the plant belong to the class of constituents called as the phenolic acids, which represent a diverse group that includes hydrobenzoic and hydrocinnamic acid (Peter and Dosa, 2002; Li et al., 2004). Many of these polyphenols are known for their antioxidant, anti-inflammatory and anticancer properties which had led to the attention in drug development using these polyphenols.

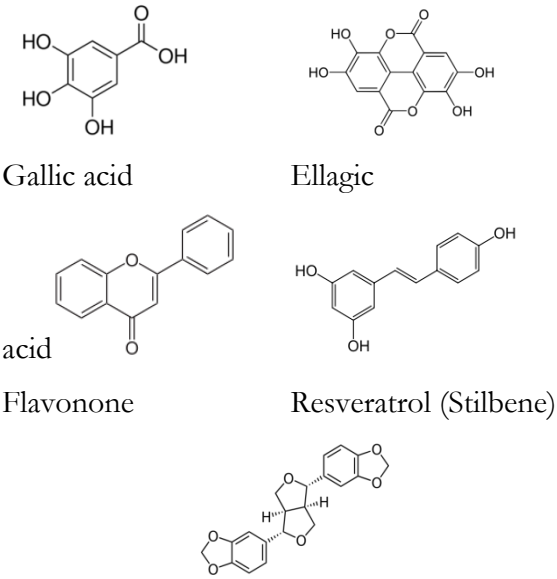


Figure 1: Some common polyphenols founds in plants

Gallic acid (3,4,5-trihydroxybenzoic acid) is a prominent endogenous plant polyphenol found in abundance in tea, grapes, berries, coffee as well as in wine (Singh et al., 2004). It occurs as yellowish-white crystal with melting point of 250°C and has a molecular weight of 170.12 g/mol). It has a water solubility of 1.1% at 20°C (Polewski et al., 2002). Gallic acid has been isolated from the rind, stem, seed, leaves and bark of many plants using water,

methanol and ethanol for extraction of the phytochemical (Mamdouh et al., 2012).

Gallic acid and its naturally occurring derivatives are known to modulate many of the physiological processes and are pharmacologically effective in many conditions as antioxidant (Kim et al., 2002), anti-inflammatory (Kroes et al., 1992), and anticancer agent (Inoue et al., 1995). Most of the pharmacological activities (therapeutic and preventive) of gallic acid and its derivatives are in diseases where oxidative stress has been implicated, including cardiovascular diseases, neurodegenerative disorders, aging and cancer (Kaur et al., 2009).

Several synthetic derivatives of gallic acid have been prepared and reported to possess a number of biological and pharmacological properties. Some of the synthetic derivatives of gallic acid are reported to be neuroprotective (Locatelli et al., 2013), inducers of cell apoptosis (Saeki et al., 2000), inhibitors of squalene epoxidase (Abe et al., 2000) and cell signaling pathway modulators (Sakaguchi et al., 1998).

Gallic acid and its naturally occurring derivatives have been isolated from various plant sources and evaluated for their pharmacological potential against diverse disease states. Sheetal et al., 2007 reported the isolation and quantification of gallic acid, gallicin, lupeol and β-sitosterol from the whole plant of Bergia suffruticosa.

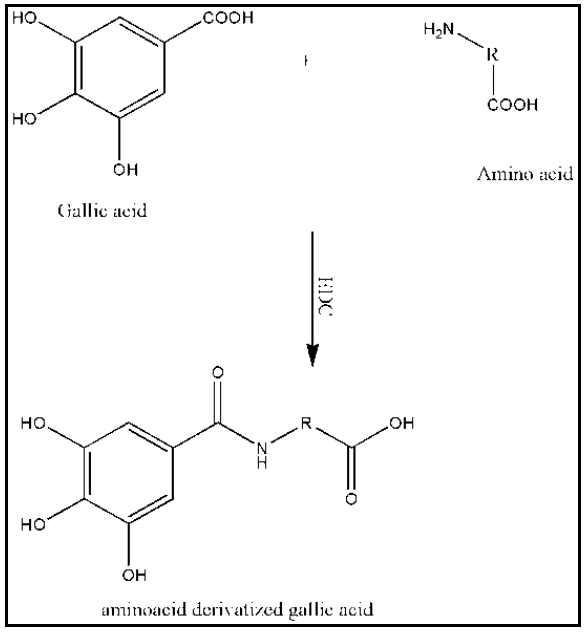
Alkyl esters of gallic acid were reviewed for their anticancer, antioxidant and neuroprotective effects (Locatelli et al., 2013). A few derivatives lipophilic derivative of gallic acid were synthesized and evaluated for their ability to induce apoptosis in cancerous cells (Saeki et al., 2000). The in vitro antiproliferative potential of a series of gallic acid derivatives bearing carbohydrazide and 1,3,4-oxadiazole was reported (Maximo da Silva et al., 2015).

In light of these facts and based on the figures and data obtained from the literature, it was thereby envisioned that by synthesizing of some amino-acid based derivatives of gallic acid it would be possible to increase the antioxidant potential of gallic acid thereby increasing the possibilities of obtaining better anticancer and anti-alzheimer compounds.

Materials and Methods

The melting points of the synthesized compounds were determined by open capillary method and are uncorrected. Solubility of the compounds was determined in various polar and non-polar solvents and the structures of the synthesized compounds were confirmed by spectral studies.

The target compounds were synthesized according to the scheme 1



Amino acids used: Glutamic acid, aspartic acid, cysteine, methionine, glutathione and phenyl alanine

Scheme 1: Synthesis of aminoacid derivatized gallic acid

Synthesis of L-amino acid derivatives of Gallic acid (Mishra et al., 2015)

Gallic acid (0.01 mol) in ethanol was mixed with 20 mg of EDC added over a period of 15 minutes with stirring. The mixture was allowed to react for 20 min at room temperature while stirring. To this mixture was added appropriate L-amino acid (0.01 mol) with continuous stirring and the reaction was continued for 6-11 h at room temperature. After the completion of reaction (as monitored by TLC), the reaction mixture was filtered and dried. The product obtained was recrystallized from methanol to yield compound.

DPPH free radical scavenging activity

In order to measure the DPPH scavenging activity, 0.2 mL of different concentrations (10, 20, 30, 40 and 50 μg/mL) of the synthesized compounds was prepared in methanol and mixed with 2 mL of DPPH solution (0.05mM). After 30 min, the absorbance of the test solutions was measured at 517 nm and the percentage of DPPH radical scavenging was calculated using the formula given below:

$$\% \text{ inhibition of DPPH radical} = ([A_{blank} - A_{test}]/A_{blank}) \times 100$$

where A_{blank} is the absorbance of DPPH solution and A_{test} is the absorbance 30 min after addition of the test solution.

IC50 value to the test compounds was determined from the percentage inhibition and reported.

Hydrogen peroxide scavenging (H_2O_2) assay

The ability of the compounds to act as antioxidant by virtue of their H_2O_2 scavenging has been performed as per the method reported by Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4) and the concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Test compounds (10, 20, 30, 40 and 50 μg/mL), in methanol were added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen

peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = ([A_{blank} - A_{test}]/A_{blank}) \times 100$$

where A_{blank} is the absorbance of control solution and A_{test} is the absorbance 10 min after addition of the test solution.

IC50 value to the test compounds was determined from the percentage inhibition and reported.

Results and Discussions

Synthesis of the desired compounds was carried out by utilizing the optimized scheme. The R_f value obtained from TLC, melting point and percent yield of the synthesized compounds is depicted in Table 1.

Solubility characteristics, percent nitrogen content and molecular weight (calculated) of the synthesized compounds are listed in Table 2.

L-Cysteine derivative of Gallic acid: ^{1}H-NMR ($CDCl_3$, 300 MHz) - δ 7.263 (CH benzene), 6.949 (OH, aromatic), 8.720 (NM amide), 11.382 (OH, carboxyl), 2.076 (CH_2), 1.511 (SH)

Mass (m/e) – 273.23 (M^+).

IR (KBr, cm^{-1}) – 3039.13 (CH st), 1393.03 (C-N st), 1215.05 (C-O st), 1718.69 (C=O st), 3554.99 (NH st), 2309.92 (SH st), 698.45 (C-S bend), 3733.07 (OH st), 1584 (C-C aromatic st)

L-Methionine derivative of Gallic acid: ^{1}H-NMR ($CDCl_3$, 300 MHz) - δ 7.02 (CH benzene), 8.207 (NH amide), 4.17 (CH methine), 2.377 (CH_2), 10.610 (OH, carboxyl), 2.033 (CH_3)

Mass (m/e) – 301.32 (M^+).

IR (KBr, cm^{-1}) – 3042.73 (CH st), 1267.31 (C-N st), 1193.84 (C-O st), 1676.80 (C=O st), 3397.17 (NH st), 2310.27 (SH st), 744.65 (C-S bend), 3732.67 (OH st), 1510.55 (C-C aromatic st)

L-Glutamatic acid derivative of Gallic acid: ^{1}H-NMR ($CDCl_3$, 300 MHz) - δ 7.004 (CH benzene), 6.013 (OH aromatic), 8.000 (NH amide), 4.494 (CH methine), 2.093 (CH_2, 10.418 (OH, carboxyl)

Mass (m/e) – 299.23 (M^+).

IR (KBr, cm^{-1}) – 3045.31 (CH st), 1325.25 (C-N st), 1223.36 (C-O st), 1715.99 (C=O st), 3112.15 (NH st), 3733.71 (OH st), 1581.55 (C-C aromatic st)

L-Aspartate derivative of Gallic acid: ^{1}H-NMR ($CDCl_3$, 300 MHz) - δ 6.696 (CH benzene), 9.891 (NH amide), 4.222 (CH methine), 10.439 (OH, carboxyl)

Mass (m/e) – 271.18 (M^+).

IR (KBr, cm^{-1}) – 2933.32 (CH st), 1284.08(C-N st), 1225.59 (C-O st), 1703.85 (C=O st), 3232.34 (NH st), 3732.59 (OH st), 1514.07 (C-C aromatic st)

L-Phenyl alanine derivative of Gallic acid: ^{1}H-NMR ($CDCl_3$, 300 MHz) - δ 7.051 (CH benzene), 8.111 (NH amide), 2.925 (CH_2), 10.416 (OH, carboxyl), 7.162-7.861 (CH benzene, PLA)

Mass (m/e) – 317.29 (M^+).

IR (KBr, cm^{-1}) – 2992.19 (CH st), 1299.77 (C-N st), 1233.37 (C-O st), 1726.28 (C=O st), 3099.37

(NH st), 3733.58 (OH st), 1527.62 (C-C aromatic st)

Antioxidant Potential

The result obtained from the antioxidant evaluation of the synthesized compounds against DPPH and H_2O_2 is depicted in Table 3 and 4 respectively

Table 1 List of the synthesized compounds

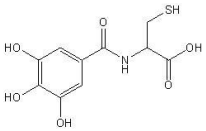
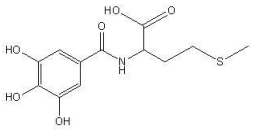
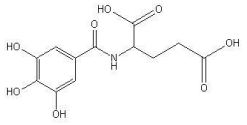
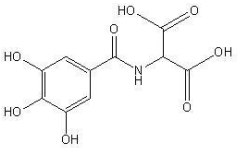
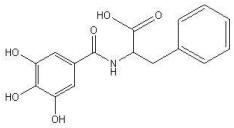
Compound Code	Structure	R_f Value	Melting Point (°C)	Yield (%)
3		0.68	228-230	48
5		0.81	276-278	72
7		0.75	221-223	64
9		0.67	240-243	56
11		0.70	268-270	67

Table 2 Physical properties of the synthesized compounds

Compound Code	Molecular Formula	Molecular Weight (calculated)	Percent Nitrogen	Color and character	Solubility
3	$C_{10}H_{11}NO_6S$	273.03	5.13	Pale yellow powder	Water, Methanol, Chloroform
5	$C_{12}H_{15}NO_6S$	301.06	4.65	Yellowish crystals	Water, Methanol, Chloroform
7	$C_{12}H_{13}NO_8$	299.06	4.68	Brown crystals	Water, Methanol, Chloroform
9	$C_{10}H_9NO_8$	271.03	5.17	Yellow powder	Water, Methanol, Chloroform
11	$C_{16}H_{15}NO_6$	317.09	4.41	Yellow powder	Chloroform

Table 3 Antioxidant potential of the synthesized compounds against DPPH

Compound Name	IC_{50} value of the test compounds
3	8.2 ± 0.2
5	12.1 ± 0.2
7	26.4 ± 0.5
9	38.0 ± 0.3
11	44 ± 0.2
Gallic Acid	13.5 ± 0.1

References

1. Abe I, Seki T and Noguchi H (2000). Potent and selective inhibition of squalene epoxidase by synthetic gallic esters. Biochemical and Biophysics Research Communication, 270, 137- 140.
2. Ahmad I, Aqil F and Owais M (2006). Modern Phytomedicine: Turning Medicinal Plants into Drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
3. Hack- Seang K (2005). Do not put too much value on conventional medicine. Journal of Ethnopharmacology, 100, 37-39
4. Inoue M, Suzuki R, Sakaguchi N, Li Z, Takeda T, Ogihara Y, Jiang B and Chen Y (1995). Selective induction of cell death in cancer cells by gallic acid. Biological and Pharmaceutical Bulletin, 18, 1526–1530.
5. Kaur M, Velmurugan B, Rajamanickam S, Agarwal R and Agarwal C (2009). Gallic acid, an active constituent of grape seed extract, exhibits antiproliferative, pro apoptotic and anti-tumorigenic effects against prostate carcinoma xenograft growth in nude mice. Pharmaceutical Research, 26, 2133–2140.
6. Kim DO, Lee KW, Lee HJ and Lee CY (2002). Vitamin C equivalent antioxidant capacity of phenolic phytochemicals. Journal of Agriculture and Food Chemistry, 50, 3713–3717.
7. Kroes BH, Van den Berg AJJ, Quarles van Ufford HC, Van Dijk H and Labadie RP (1992). Anti-inflammatory activity of gallic acid. Planta Medica, 58, 499–504.
8. Li N, Ning-hua T and Zhou J (2004). New chlorine-containing phenoloid from Curculigo capitulata. Journal of Asian Natural Product Research, 6, 7–10.
9. Locatelli C, Filippin MFB and Creczynski PTB (2013). Alkyl esters of gallic acid as anticancer agents: a review. European Journal of Medicnal Chemistry, 60, 233-239.
10. Mamdouh SM, Sawsan SH, Alaa EA and Nessma MN (2012) Synthesis and spectroscopic characterization of gallic acid and some of its azo complexes. Journal of Molecular Structure, 1014: 17-25.
11. Maximo da Silva M, Comin M, Duarte TS, Foglio MA, de Carvalho JE, et al. (2015). Synthesis, Antiproliferative Activity and Molecular Properties Predictions of Galloyl Derivatives. Molecules, 20, 5360-5373
12. Mishra BJ, Kaul A and Trivedi T (2015). L-Cysteine conjugated poly L-lactide nanoparticles containing 5-fluorouracil: formulation, characterization, release and uptake by tissues in vivo. Drug Delivery, 22 (2), 214-222
13. Peter A and Dosa G (2002). Detection of phenoloids in some Hungarian inula and

Centaurea species. Acta Botanica Hungaria, 44, 129–135.

14. Plotkin MJ (1991). Traditional Knowledge of Medicinal Plants- the search for new jungle medicine. Cambridge University Press, Cambridge UK. 245-246.
15. Polewski K, Kniat S and Slawinska D (2002). Gallic acid, a natural antioxidant, in aqueous and micellar environment: spectroscopic studies. Current Topics in Biophysics, 26, 217–227.
16. Saeki K, You A, Isemura M, Abe I, Seki T, et al. (2000). Apoptosis inducing activity of lipid derivatives of gallic acid. Biological and Pharmaceutical Bulletin, 23, 1391-1394.
17. Sakaguchi N, Inoue M and Ogihara Y (1998). Reactive oxygen species and intracellular Ca2+, common signals for apoptosis induced by gallic acid. Biochemical Pharmacology, 55, 1973-1981.
18. Sheetal A, Honnegowda S and Mandapati R (2007). Isolation and TLC Densitometric Quantification of Gallicin, Gallic Acid, Lupeol and β-Sitosterol from Bergia suffruticosa, a Hitherto Unexplored Plant. Chromatographia, 66, 725-734.
19. Singh J, Rai GK, Upadhyay AK, Kumar R and Singh KP (2004). Antioxidant phytochemicals in tomato (Lycopersicon esculentum). Indian Journal of Agricultural Science, 74, 3–5.
20. Solecki R (1977). A Neanderthal flower burial in northern Iraq. Science, 190,880-881.