



## 3,4,5-TRIHIDROXYCYCLOHEXYL METHANOL - A NEW REDUCED DERIVATIVE FROM STRUCTURAL ACTIVITY RELATIONSHIP STUDIES (SARS) ON GALLIC ACID

Olawale H. Oladimeji<sup>[a]\*</sup>, Magdalene A. Anwana<sup>[a]</sup>, Emmanuel E. Attih<sup>[a]</sup> and Daniel E. Effiong<sup>[b]</sup>

**Keywords:** Gallic acid; esterification; reduction; ethyl gallate; 3,4,5-trihydroxycyclohexyl methanol.

Gallic acid or 3,4,5-trihydroxybenzoic acid is a poly-hydroxyl compound with potential therapeutic effects in the treatment/management of oxidative stress implicated in cancers, cardiovascular and neurodegenerative diseases amongst many others. The identity, purity, integrity and suitability of the acid were ascertained and established prior to the preparation of derivatives. Furthermore, a simple titrimetric method for its assay was designed. The esterification and selective reduction of the acid led to two derivatives coded ME and MA whose identities have been established to be ethyl gallate and 3,4,5-trihydroxycyclohexylmethanol (possibly a new reduction derivative) respectively using the IR spectral technique. Gallic acid and MA demonstrated minimal antioxidant activity at  $IC_{50}$  of 0.76 and 0.89  $\mu\text{g mL}^{-1}$ , respectively. However, ME was remarkably active at 0.37  $\mu\text{g mL}^{-1}$  which compare favourably with 0.34  $\mu\text{g mL}^{-1}$  elicited by Vitamin C (a standard antioxidant drug). The obtained results indicate that esterification enhances the antioxidant activity of gallic acid.

### \* Corresponding Authors

Tel.: +2348146306558; +2347038916740

E-Mail: wale430@yahoo.co.uk;

hakeemoladmeji@uniuyo.edu.ng

[a] Department of Pharmaceutical & Medicinal Chemistry,  
Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

[b] Department of Pharmaceutics & Pharmaceutical Technology,  
Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

## INTRODUCTION

Free radicals which can be reactive oxygen species (ROS), reactive nitrogen species (RNS) and many others are atoms, molecules, or ions that have unpaired valence electrons which make them chemically very reactive.<sup>1</sup> These chemical species are unstable and have short life time.<sup>1-2</sup> The ROS especially can be produced from either endogenous or exogenous sources. The endogenous sources of ROS include cellular organs/ organelles such as mitochondria, peroxisomes, endoplasmic reticulum while the exogenous sources include pollution, alcohol, tobacco smoke, heavy transition metals amongst many others.<sup>3</sup> It could be a daunting task for humans to avoid damage by free radicals. Hence, there is an everpressing need for antioxidants which are substances that when present in low concentrations compared with that of an oxidized substrate would significantly delay or prevent the oxidation of that substrate.

The oxidized substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids and proteins.<sup>4</sup> However, some of the antioxidant drugs in clinical practice are toxic, poorly active and expensive. Hence, there is need to prospect for templates with little or no toxicities, better activities and which are affordable for general people. A known chemical substance with antioxidant activity is gallic acid. It is a white crystalline substance found in gallnuts, witch-hazel tea leaves, oak bark, strawberries, grapes, bananas and vinegars<sup>5,6</sup> amongst many other plants. The name is derived from oak galls, which were historically used to prepare

tannic acid. It is found both free and as part of hydrolysable tannins. The gallic acid groups are usually bonded to form dimers such as ellagic acid. Hydrolysable tannins break down on hydrolysis to gallic acid and glucose or ellagic acid and glucose.<sup>7</sup> In addition, gallic acid has been isolated from a number of plants such as *Cynimcrum coccineum*, *Myriophyllum spicatum*, *Caesulpina nimosoida* and *Boswellia dalzielii*<sup>8-11</sup> amongst many others. The interest in this compound is due to its pharmacological activity as a radical scavenger which has been proved to have potential preventive and therapeutic effects in many diseases such as convulsion, microbial diseases, cardio-vascular diseases, cancer, neuro-degenerative disorders<sup>12-14</sup> and etc. where oxidative stress has been implicated. Consequently, gallic acid was considered as a promising lead compound for structural activity relationship studies (SARS).

In the present study, the acid was chemically modified to its ethyl ester and reduced derivatives by esterification and reduction respectively. The acid and synthesized products were tested for antioxidant activities using the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) reagent and the obtained antioxidant activities ( $IC_{50}$ ) were compared.

## EXPERIMENTAL

### Reagents/chemicals

Gallic acid and DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) were obtained from Tianjin Kernel Chemical Reagent Company, China and Sigma Aldrich Chemicals, Germany, while acetic acid, chloroform, diethyl ether, dichloromethane, ethanol, hydrochloric acid, magnesium sulphate, methanol, iodine, petroleum ether, sodium borohydride, sulphamic acid, sodium hydroxide, sulphuric acid and tetrahydrofuran were obtained as AnaLAR Grade Chemicals from BDH Chemicals Limited, Poole, England. NaOH (1 M) solution used was standardized with sulfamic acid in the presence of phenolphthalein indicator.

Dissolution studies of gallic acid was done in chloroform, ethanol, dichloromethane, diethyl ether, methanol, petroleum ether and distilled water. Melting points were determined<sup>15</sup> using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England). Gallic acid content was determined by dissolution of sample in 0.5 M NaOH added, the mixture was heated in a water water-bath for 10 minutes, cooled and titrated with 0.5 M HCl in the presence of phenolphthalein as indicator.

The optical rotation and refractive index of gallic acid dissolved in ethanol were measured with using a Polarimeter type ADP-220 (Bellingham Stanley, England) and a refractometer type WAY-15 (Abbe, England) at the wavelength ( $\lambda$ ) of sodium D line (589.3 nm) at 20.5 °C. The optical rotation and refractive indices of the derivatives being liquids were measured directly without dissolution in any solvents.<sup>16</sup> IR characteristics were measured by using the FTIR 84005 Spectrophotometer (Shimadzu, Japan).

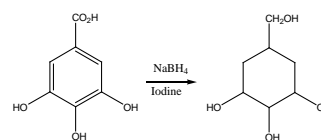
To prepare a calibration curve for DPPH reagent (2,2-diphenyl-1-picrylhydrazyl hydrate), DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions of the stock solution were carried out and the absorbance of each of the sample was taken at 512 nm using the UV spectrophotometer (Jenway 6405, USA). Methanol without DPPH was served as the blank.

#### Esterification of gallic acid

Gallic acid (m.p. 258-260 °C.  $[n]_D^{20}$  1.704,  $[\alpha]_D^{20} +38^\circ$ , FTIR: 1615 (Ar-C=C), 1705 (-C=O), 2854 (-CH stretching), 2922 (-CH stretching), 3281 (-OH) and 3361 (-OH)  $\text{cm}^{-1}$  (1.0 g) was added to 50 mL of ethanol and the mixture was stirred with a glass rod for 20 min until the particles were completely dissolved to obtain a clear solution. To the solution in the flask, another 50 mL of ethanol was added to ensure complete dissolution of the particles. Concentrated sulphuric acid (10 mL) was added to the solution which served as a catalyst. The flask containing the solution was corked with aluminum foil to prevent air. The reacting mixture was kept for two weeks in a refrigerator at 4 °C to ensure complete esterification.<sup>17</sup> Ethyl gallate (ME) was given as yellow liquid.  $[n]_D^{20}$  1.730,  $[\alpha]_D^{20} +46^\circ$ ,  $d=1.73$   $\text{g cm}^{-3}$ . FTIR: 1037 (C-O-C, ether linkage), 1633 (Ar-C=C), 1715 (C=O), 3440 (-OH) and 3851 (-OH)  $\text{cm}^{-1}$ .

#### Reduction of gallic acid

Gallic acid (1.5 g) was dissolved in 20 mL of THF. The resultant solution was slowly added to a suspension of  $\text{NaBH}_4$  (0.45 g) in 200 mL of THF at room temperature for 10 min. The mixture was stirred until evolution of gas ceased. 0.63 g of iodine and 20 mL of THF were carefully added to the mixture immersed in an ice-bath with the evolution of more gas. The mixture was further stirred for 1 h. Dilute HCl (5 mL) was added carefully and the mixture extracted several times with ether. The combined ether extract was washed with 3 M NaOH (30 mL), brine and dried over anhydrous  $\text{MgSO}_4$ . Evaporation of the organic layer gave the reduced product.<sup>18</sup>



**Scheme 2.** Reduction of gallic acid.

3,4,5-Trihydroxycyclohexyl methanol (MA) was given as colourless liquid.  $[n]_D^{20}$  1.6111,  $[\alpha]_D^{20} +30^\circ$ ,  $d=1.30$   $\text{g cm}^{-3}$ . FTIR: 2922 (CH stretching) and 3437 (-OH)  $\text{cm}^{-1}$ .

#### Antioxidant activity

Substances which are capable of donating electrons or hydrogen atoms can convert the purple-colored DPPH radical (2,2-diphenyl-1-picrylhydrazylhydrate) to its yellow-colored non-radical form, 1,1-diphenyl-2-picrylhydrazine.<sup>19,20</sup> This property was used to determine the antioxidant activity of derivatives of gallic acid and vitamin C. To determine the antioxidant activity of gallic acid, derivatives and vitamin C, 2 mg or 2 mL (liquids derivatives) each of sample was dissolved in 50 mL of methanol. Serial dilutions were carried out and 5 mL of each solution was incubated with 5 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 min in the dark at room temperature ( $25 \pm 2$  °C), observation was made for a change in the color of the mixture from purple to yellow. The absorbance of each of the test samples was then taken at 512 nm. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

$$\text{RSA \% (PI \%)} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100 \quad (1)$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction (DPPH solution without the test sample) and  $A_{\text{sample}}$  is the absorbance of DPPH incubated with the sample. Vitamin C was used as a standard antioxidant drug. MGA/ME/MA/Vitamin C concentration providing 50 % inhibition ( $IC_{50}$ ) was calculated from a graph of inhibition percentage against the concentration of the MGA /ME/MA/Vitamin C.<sup>22-23</sup>

#### Antioxidant activity

A calibration curve was prepared for DPPH reagent with an aim of confirming its purity, integrity and suitability for use in the antioxidant determinations. The Beer-Lambert's Law is the basis of all absorption spectrophotometry. Therefore, a plot of absorbance against concentration for a cell of unit thickness should give a linear graph. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of 512 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (MGA/ME/MA/Vitamin C) increased which suggested that the DPPH reagent was being reduced. Table 1 shows radical scavenging activity (RSA %) or percentage inhibition (PI %) and the computed values of  $IC_{50}$  of MGA, ME, MA and Vitamin C.

## RESULTS AND DISCUSSION

Gallic acid employed in this study was put through some monographic determinations with a view to ascertaining and establishing its identity, purity, integrity and suitability. Hence, its solubility profile and melting point determination were carried out. The acid was soluble in all the organic solvents used except petroleum ether while its melting point was observed 258-260 °C. These observed results are comparable with standard values in the literature. Prior to this study, there was no titrimetric method for assaying gallic acid. Hence, its assay was carried out using NaOH solution. Furthermore, the technique known as back-titration was used because direct titration of an acid against an alkali may sometimes be difficult. Partial hydrolysis could take place leading to the formation sodium gallate as a side product. Consequently, the excess NaOH was titrated against HCl. Also, a blank titrimetric determination was done without the sample of gallic acid. The percentage purity of the sample of gallic acid was computed to be 98.60 % w/w. Gallic acid gave a refractive of 1.704 and demonstrated an optical rotation of +38°. Diagnostic IR peaks at 3361, 3281, 2922, 2854, 1705 and 1615 cm<sup>-1</sup> indicate the characteristic -OH, -CH, -C=O and Ar-C=C stretchings respectively as can be seen in the IR spectrum of MGA.

Ethyl gallate (ME) was synthesized as a yellow liquid with a pleasant fruity smell which is characteristic of esters. This derivative showed a refractive index of 1.730 and gave an optical rotation of +46°. In addition, the IR spectroscopic analyses of ME show peaks at 3851, 3440, 1715, 1633 and 1054 cm<sup>-1</sup> which are characteristic of -OH, -C=O, Ar-C=C (which was observed to absorb higher than that seen in MGA) and -C-O-C stretchings respectively.

MA was synthesized by the reduction method.<sup>18</sup> This procedure converts a carboxylic acid to an alcohol using sodium borohydride. This derivative gave a refractive index of 1.611 and an optical rotation of +30°. IR peaks at 3437 and 2922 cm<sup>-1</sup> reveal the OH stretching and -CH absorptions respectively as are found in the IR spectrum of MA. It is instructive to state that this reduction method was primarily supposed to selectively reduce the -C=O group of the COOH to a -CH<sub>2</sub> (methylene). Furthermore, in addition to the removal of the -C=O peak at 1705 cm<sup>-1</sup>, the IR spectrum also shows that the 3 endocyclic aromatic C=C bonds represented by the peak at 1615 cm<sup>-1</sup> disappeared indicating that the aromaticity of the gallic acid had been lost. This indicates that saturation of the three C=C bonds had taken place. Comprehensive database search of organic compounds was carried out and no piece of information or data about 3,4,5-trihydroxycyclohexyl methanol were

obtained. Hence, it could be safe to infer that the compound is probably a new derivative of the reduction of gallic acid.

It is interesting to note that gallic acid and its derivatives showed optical rotations of +38°, +46° and +30° respectively, indicating that the three compounds are optically active. All these compounds rotate plane of polarized light in the clockwise directions. Hence, the compounds can demonstrate dextrorotation.

In antioxidant studies, the RSA % is an indicator of the antioxidant activity of MGA / ME/ MA/ vitamin C. Both gallic acid and MA demonstrated marginal antioxidant activity, IC<sub>50</sub> of 0.76 and 0.89 μg mL<sup>-1</sup> respectively compared with ethyl gallate which was remarkably active at 0.37 μg mL<sup>-1</sup>. Furthermore, the activity recorded by ME was not surprising because this compound is highly lipophilic due to the presence of the ethyl group moiety which enables it transverse the lipid membrane much easily and readily to the allosteric (active) sites better and faster than gallic acid to effect the pharmacological action of anti-oxidation. It must be stated that the ester showed an IC<sub>50</sub> close to that shown to vitamin C (standard antioxidant drug) at 0.34 μg mL<sup>-1</sup>. Interestingly, ethyl gallate isolated from *Acalypha wilkesiana* var. *laceacalypha* (Muell and Arg.) was observed to possess antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*.<sup>23</sup>

## CONCLUSION

This present study reports the synthesis of a probably new derivative from the reduction of gallic acid with a marginal (minimal) antioxidant activity. However, the esterification of gallic acid enhances its antioxidant activity as the ester obtained therefrom demonstrated an antioxidant activity which compare favourably with the activity elicited by a standard antioxidant drug, Vitamin C.

## ACKNOWLEDGEMENTS

The authors are grateful for the kind gesture of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo for the use of its Jenway 6405UV/VS Spectrophotometer in the antioxidant assays. We also acknowledge the contribution of the University of Ibadan, Nigeria in obtaining the IR spectra with the use of their facilities.

**Table 1.** Radical Scavenging activity (Percentage Inhibition) of samples at different concentrations and IC<sub>50</sub> of the compounds.

Sample	Concentration, mg mL <sup>-1</sup>					IC <sub>50</sub> μg mL <sup>-1</sup>
	0.0004	0.0008	0.0012	0.0016	0.0020	
MGA	36.16	57.55	58.78	59.18	78.98	0.76
ME	81.22	81.62	82.64	83.27	84.13	0.37
MA	38.35	38.78	57.95	58.10	58.98	0.89
Vitamin C	88.10	88.47	88.65	89.28	89.68	0.34

## REFERENCES

- <sup>1</sup>Hayyan, M., Hashim, M., Alnashef, I., Superoxide ion: Generation and chemical implications, *Chem. Rev.*, **2016**, *116*(5), 3029-3085. DOI: 10.1021/acs.chemrev.5b00407.
- <sup>2</sup>Cheeseman, K., Slater, T., An introduction to free radicals chemistry, *Brit. Med. Bull.*, **1993**, *49*(3), 481-493. DOI: org/10.1093/oxfordjournals.bmb.a072625.
- <sup>3</sup>Speed, N., Blair, I., Cyclooxygenase and lipoxygenase-mediated DNA damage, *Cancer Metastasis Rev.*, **2011**, *30*(34), 437-472. DOI: org/10.1007/s10555-011-9298-8.
- <sup>4</sup>Wanasundara, P., Shahidi, F., *Antioxidants: Science, Technology and Applications. Bailey's Industrial Oil and Fat Products*, **2005**, John Wiley and Sons, Inc., 1-11. ISBN: 9780471678496, DOI: 10.1002/047167849X
- <sup>5</sup>Hodgson, J., Morton, L., Puddy, I., Beilin, L., Croft, K., Gallic acid metabolites are markers of black tea intake in humans, *J. Agric. Food Chem.*, **2000**, *48*(6), 2276-2280. DOI: org/10.1021/Jf0000895.
- <sup>6</sup>Pathak, S., Niranjana, K., Padh, H., Rajani, M., TLC densitometric method for the quantification of eugenol and gallic acid in clove, *Chromatography*, **2004**, *60*(3), 241-244. DOI: 10.1365/510337-004-0373-y.
- <sup>7</sup>Andrew, J., Availability of non-carotenoid antioxidants affects the expression of a carotenoid-based sexual ornaments, The role of antioxidants in photo-protection, *J. Am. Acad. Dermatol.*, **2004**, *67*(5), 1013-1024. DOI: 10.1016/j.jacid.2019.12.077.
- <sup>8</sup>Nakai, S., *Myriophyllum spicatum*-released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*, *Water Res.*, **2000**, *34*(11), 3026-3032. DOI: org/10.1016/S0043-1354(00)00039-7.
- <sup>9</sup>Alemika, T. E., Onawunmi, G. O., Olugbade, T. A., Isolation and characterization of incense from *Boswellia dalzielii* stem bark, *J. Pharm. Biores.*, **2004**, *1*(1), 7-11. DOI: 10.4314/jpb.ril.32041.
- <sup>10</sup>Chanutheesuk, A., Teerawutgulrag, A., Kilburn, J., Rakariyatham, N., Antimicrobial gallic acid from *Caesalpinia minosoides* bark, *Food Chem.*, **2007**, *100*(3), 1044-1048. DOI: org/10.1016/j.foodchem.2020.126226.
- <sup>11</sup>Zucca, P., Antonella, R., Carlo, T., Rinaldi, A., Enrico, S., Maria, D., Antonio, R., Evaluation of antioxidant potential of Maltese mushroom (*Cynomorium coccincum*) by means of multiple chemical and biological assays, *Nutrients*, **2013**, *5*(1), 149-161. DOI : org/10.3390/nu5010149.
- <sup>12</sup>Duibedy, B., Gyamfi, A., Yonamine, Y., Aniya, Y., Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries, *Gen. Pharmacol.*, **1999**, *32*(6), 661-667. DOI: org/10.1016/S0306-3623(98)00238-9.
- <sup>13</sup>Anurag, R. Allison, M., Lackey, L., McDougale, R., Brown, W., Law, E., Harris, R., Temiz, N., Refls, E., Kotandeyya, D., Nikas, B., Birns, M., Carpenter, M., Tretyakova, W., Temic, N., APOBEC3B is an enzymatic source of mutation in breast cancer, *Nature*, **2013**, *494*(7437), 366-370. DOI: 10.1038/nature11881.
- <sup>14</sup>Locatelli, C., Branco, F., Creczynski-Pasa, T., Alkyl esters of gallic acid as anticancer agents, *Eur. J. Med. Chem.*, **2013**, *60*, 233-239. DOI: org/10.1016/j.ejmech.2012.056.
- <sup>15</sup>*British Pharmacopeia, Appendix VA, A109*, The Pharmaceutical Press London, **1993**, 754.
- <sup>16</sup>Olaniyi, A. A., *Principles of quality assurance and pharmaceutical analysis*, Mosuro Publishers, **2000**.
- <sup>17</sup>Morrison, R.T, Boyd, R.N, *Organic chemistry*, Prentice-Hall Inc., New York, **1973**, 602-603; 672-673.
- <sup>18</sup>Bhaskar-Kanth, J. V., Periasamy, M., Selective reduction of carboxylic acids into alcohols using NaBH<sub>4</sub> and I<sub>2</sub>, *J. Org. Chem.*, **1991**, *56*(20), 5964-5965. DOI:10.1021/jo00020a052.
- <sup>19</sup>Nagalapur, S. K., Paramjyothi, S., *In-vitro* antioxidant activity of *Launaea pinnatifida* Cass leaves, *The Bioscan*, **2010**, *5*(1), 105-108. www.thebioscan.in /journals/abs2010.5nl\_105\_108.htm.
- <sup>20</sup>Guangrong, H., Jiabin, J., Dehui, D., Anti-oxidative and antibacterial activity of the methanol extract of *Artemisia anomala* S. Moore, *Afr. J. Biotech.*, **2008**, *7*(9), 1335-1338. DOI: 10.1011060.8245 and rep.repl.
- <sup>21</sup>Lebeau, J., Furman, G., Bernier, J. L., Durietz, P., Teissier, E., Cotelle, N., Antioxidant properties of di-tert-butylhydroxylated flavonoids. *Free-Radical Biology and Med.*, **2000**, *29*(9), 900-912. [https://doi.org/10.1016/S0891-5849\(00\)00390-7](https://doi.org/10.1016/S0891-5849(00)00390-7).
- <sup>22</sup>Leitao, G. G., Leitao, S. G., Vilegas, W., Z. Quick preparative separation of natural naphthoquinones with antioxidant activity by high speed counter current chromatography, *Z. Naturforsch.*, **2002**, *57C*, 1051-1055. <https://doi.org/10.1515/znc-2002-11-1217>.
- <sup>23</sup>Oladimeji H. O., Tom, E. U., Attih, E. E., Ethyl gallate and Pyrogallol from *Acalyphawilkesiana* var. laceacalypha (Muell & Arg.), *Eur. Chem. Bull.*, **2014**, *3*(8), 788-791. DOI: 10.17628/ecb.2014.3.788.

Received: 22.01.2020.

Accepted: 11.03.2020.