EVALUATION OF ANTIOXIDANT ACTIVITY OF CINNAMIC ACID AND SOME OF ITS DERIVATIVES

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Keywords: Cinnamic acid; esterification; selective-reduction; ethyl cinnamate; cinnamyl alcohol.

Cinnamic acid, chemically known as 3-phenyl-2-propenoic acid is has a broad spectrum of pharmacological actions including antioxidant activity. The esterification and selective reduction of the acid led to ethyl cinnamate and cinnamyl alcohol, respectively. Cinnamic acid demonstrated a poor antioxidant activity (IC_{50}) of 1.2 µg mL^{-1} while ethyl cinnamate and cinnamyl alcohol elicited activities of 0.64 µg mL^{-1} (moderate) and 0.84 µg mL^{-1}, respectively. The obtained results indicate that esterification enhances the antioxidant activity of cinnamic acid.

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INTRODUCTION

Free-radicals are chemical species which have been implicated as the causative agents in many degenerative conditions such as Alzheimer's and Parkinson's disease, stroke, cancer, pancreatitis, laryngitis, asthma, hay fever, rheumatoid arthritis, wounds, atherosclerosis, emphysema, lungs dysfunction, radiation injuries, premature aging and diabetes amongst many others.[1-4] Living organism possesses antioxidant defense and repair system which offer some protections against oxidative stress but nevertheless are insufficient to prevent the damage. Antioxidants are believed to play an important role in the body defence system against radical oxidative species (ROS)[5-6] and at low concentrations significantly delay or inhibit oxidation of body tissues.[7]

Some of the antioxidant drugs in clinical practice are costly, toxic and poorly active. Hence, there is need to search for templates with little or no toxicities. A known chemical substance with antioxidant activity, cinnamic acid was considered. It is a white colored crystalline organic acid occurring naturally in plants which has low toxicity and a broad spectrum of biological activities. This acid is used as a flavoring agent in certain pharmaceuticals and in the manufacture of the esters for the perfume and fragrance industry. Cinnamic acid can being structurally modified into compounds with probable potentials for higher antioxidant activities. In the present study, the acid was chemically modified to its ethyl ester and reduced derivatives by esterification and $NaBH_4$-reduction (selective reduction) respectively. The acid and synthesized products were screened for antioxidant activities using the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) reagent and the obtained antioxidant activities (IC_{50}) compared.

EXPERIMENTALS

Cinnamic acid and DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate**)** were sourced from Sigma Chemicals, Germany) while acetic acid, acetone, benzene, chloroform, diethyl ether, ethanol, hydrochloric acid, magnesium sulphate, methanol, petroleum ether, sodium borohydride, sodium hydroxide, sulphuric acid and tetrahydrofuran were obtained as AnaLAR Grade Chemicals from BDH Chemicals Limited Poole, England.

Melting points were determined using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England).[8] The properties of the cinnamic acid and derivatives are the followings:

Cinnamic acid: $C_9H_8O_2$; mol. wt. (148.16 g/mol); white color crystalline solid; m.pt. (132-134 ^{0}C); $[n]_D^{20}$ (1.616); $[\alpha]_D^{20}$ (0^0); [d] (1.25 g /cm^3): FTIR (cm^{-1}): 1576 (Ar-C=C), 1627 (acyclic C=C), 1682 (α, β unsaturated C=O), and 2923 (-OH).

Ethyl cinnamate: $C_{11}H_{12}O_2$; mol. wt. (176.21 g/mol); pale-yellow liquid; $[n]_D^{20}$ (1.658); $[\alpha]_D^{20}$ (0^0); [d] (1.05 g /cm^3): FTIR (cm^{-1}): 711, 767 (CH_2 and CH_3 bending modes), 1037 (C-O-C, ether linkage), 1637 (Ar-C=C) and 1730 (C=O).

Cinnamyl alcohol: $C_9H_{10}O_1$; mol. wt. (134.16 g/mol); colorless liquid; $[n]_D^{20}$ (1.581); $[\alpha]_D^{20}$ (0^0); [d] (1.04 g /cm^3): FTIR (cm^{-1}): 754, 1631 (Ar-C=C), 1681 (acyclic C=C) and 3200 (-OH).

Assays

Cinnamic acid (1.5 g) was carefully weighed out into a conical flask and 50 mL of 0.5 M NaOH added. The mixture was immersed in boiling water bath for 10 minutes, cooled and three drops of phenolphthalein were added as indicator. Titration was then carried out against the excess alkali using 0.5 M HCl and the end-point noted (pink color). Blank titration was carried out without the sample.

Ethyl cinnamate synthesis[9]

Cinnamic acid (1.0 g) was dissolved in 100 mL of ethanol and 10 mL of cc, sulphuric acid was added. The flask was corked with Al foil and kept for 2 weeks in a refrigerator at 4 ^{0}C to ensure complete synthesis of the ester.

Selective reduction of cinnamic acid[10]

Cinnamic acid (1.5 g) was dissolved in 20 mL of THF and this solution was slowly added to a suspension of $NaBH_4$ (0.45 g) in 200 mL of THF at room temperature in 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 with ether. The combined ethereal extracts was washed with 3 M NaOH (30 mL), brine and dried over $MgSO_4$. Evaporation of the organic layer gave the reduced product.

Determination of optical rotation and refractive index

Cinnamic acid was dissolved methanol and the optical rotation was measured at 589.3 nm at 20.5 ^{0}C with using an ADP-220 (Bellingham Stanley, England) polarimeter. The refractive indexes were measured on a WAY-15 (Abbe, England) refractometer at the same wavelength and temperature.[11-12] The optical rotation and refractive indices of the derivatives being liquids were measured directly without dissolution in any solvents.

Spectrophotometric determination of antioxidant activity using DPPH reagent

Substances which are capable of donating electrons or hydrogen atoms can convert the purple-colored DPPH radical (2,2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-colored non-radical form; 1,1-diphenyl-2-picrylhydrazine.[13-14] The absorbance of each sample was taken at λ_m 512 nm using a Jenway 6405 (USA) UV-spectrophotometer.

Determination of the antioxidant activity of cinnamic acid, its derivatives and vitamin C

Each of sample (2 mg) was dissolved in 50 mL of methanol. Serial dilutions were carried out and each concentration was incubated with same volume of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 ^{0}C), the absorbance of each test sample was then taken at λ_m 512 nm. The radical scavenging activity (RSA %) or percentage inhibition (PI %) of free radical DPPH were calculated according to th stahdard equation:

$$RSA(PI,\%) = 100\,\frac{A_{blank} - A_{sample}}{A_{blank}}$$

where A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and A_{sample} is the absorbance of DPPH incubated with the samples. Vitamin C concentration providing 50 % inhibition (IC_{50}) was calculated from a graph of inhibition percentage against the concentration of the samples and Vitamin C.[15-16]

Infrared spectroscopy

IR spectra were recorded with a Shimadzu 8400S FT-IR (Japan) spectrophotometer.

RESULTS AND DISCUSSION

Cinnamic acid used in this study was subjected to analysis for ascertaining and establishing its purity. Its melting point was found to be 132-134 ^{0}C. The percentage purity of the sample of cinnamic acid was computed to be 97.27 % w/w from the back-titration of sodium hydroxide. Its refractive index was found to be 1.616 and there was no optical rotation. Diagnostic IR stretchings at 1576, 1627, 1682 and 2923 cm^{-1} indicate the characteristic Ar-C=C, acyclic C=C, α, β unsaturated C=O and –OH groups, respectively.

Ethyl cinnamate was synthesized as a pale yellow liquid. This compound was isolated as a yellow oil from *Pycnanthus angolensis* (Welw.) Warb using column chromatography (CC) and preparative column chromatography (p TLC) and characterized by IR and GC/MS techniques.[17] Its refractive index was found to be 1.658.

Cinnamyl alcohol was synthesized by the selective-reduction method.[10] This procedure converts a carboxylic acid to an alcohol using sodium borohydride in the presence of iodine. Its refractive index was found to be 1.581.

Table 1. Radical scavenging activity (percentage inhibition) of samples at different concentrations and IC_{50} values of samples

Sample	Concentration, mg mL^{-1}					IC_{50}, µg mL^{-1}
	0.0004	0.0008	0.0012	0.0016	0.0020	
Cinnamic acid	43.28	46.78	50.98	57.98	62.18	1.2
Ethyl cinnamate	46.50	52.38	58.54	64.42	71.84	0.64
Cinnamyl alcohol	41.10	50.98	55.18	63.58	69.18	0.84
Vitamin C	87.10	87.39	87.69	88.09	88.70	0.34

Antioxidant activity

The radical scavenging activity (RSA %) or percentage inhibition (PI %) and the IC_{50} values of the studied cinnamic acid derivatives or Vitamin C are given in Table 1. Ethyl cinnamate gave a moderate antioxidant activity (IC_{50}) of 0.64 µg mL^{-1} while the activity of reduced derivative was marginal at 0.84 µg mL^{-1} Cinnamic acid was poorly active (1.2 µg mL^{-1}).

Ethyl cinnamate demonstrated a higher antioxidant activity than cinnamic acid due to its lipophilic character. Hence, ethyl cinnamate is able to transverse the lipid membrane to the allosteric (active) sites better and faster than cinnamic acid to effect the pharmacological action of anti-oxidation. Ethyl cinnamate isolated from *P. angolensis* (Welw.) Warb was found to be antioxidant at 0.48 µg mL^{-1} [18-20] while the synthesized one showed an activity of 0.64 µg mL^{-1}.

CONCLUSION

The results of this present study indicate that the esterification of cinnamic acid enhances its antioxidant activity which compare favorably with the activity elicited by a standard antioxidant drug (Vitamin C).

ACKNOWLEDGEMENTS

The authors gladly acknowledge the kind gesture of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo for the use of its Jenway 6405 UV/VIS spectrophotometer in the antioxidant assays. Also, we are grateful to the University of Ibadan, Nigeria for use of their Infra-Red Spectrophotometer (FTIR, Shimadzu, Japan) in the IR analyses.

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Received: 18.05.2019.
Accepted: 15.07.2019.