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Original Article

Novel aryl substituted alkenol (ladimejol) from the chemical modifications of caffeic acid and prospection for its potential biological activities

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(3,4-benzenediol-2-propen-3-ol)

ABSTRACT

Background: Caffeic acid is a poly-phenolic compound which can be obtained from natural sources such as coffee, turmeric, oregano, mushrooms, vegetables and fruits amongst many others. This acid and a number of its synthesized derivatives have shown different activities such as anti-diabetic, antiviral, anti-inflammatory, anti-aging, cardio-protective potentialities, antioxidant and antimicrobial amongst others. This necessitated the present study.

Objectives: Increasing concerns about the debilitating effects of free radical species have become a source of worry to man hence the attention of the scientific world. These chemical species routinely wrought damages in human cells, tissues and organs resulting in a host of diverse patho-physiological and neurodegenerative conditions. Furthermore, the growing resistance to antibiotics and antifungal drugs has encouraged the search for lead compound(s) with the aim of chemically modifying the molecular structure(s) of compounds/drugs/chemicals or synthesizing derivatives from different reactions involving them. The search for novel and pharmacologically active derivatives with the aim of reducing the effects or out-right termination of these conditions led to the choice of caffeic acid.

Methodology: The pro-drug, caffeic acid was subjected to different synthetic procedures such as esterification, acetylation and reduction. The melting points, refractive indices and optical rotations of the acid and derivatives were obtained. The antioxidant activity (IC50) of caffeic acid and synthesized products was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) test. A comparison of the obtained antioxidant activities was done to determine if any improvements could be noticed in the derivatives. The agar-in-hole method was adopted for screening caffeic acid and the synthesized compounds against Staphylococcus aureus, Escherichia coli and Candida albicans for antibacterial and antifungal activities respectively.

Results: The identities of the derivatives have been revealed to be ethyl caffeate (CE), acetyl caffeate (CA) and 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol) (CR), a novel aryl substituted alkenol assigned the trivial nomenclature of (Ladimejol) respectively using a combination of physico-chemical determinations and IR spectral technique. Caffeic acid, (CE), and (CA) demonstrated significant antioxidant activity (IC50) of 0.22, 0.19 and 0.16 µg/mL respectively which compare favourably with 0.18 µg/mL obtained with Vitamin C (a standard antioxidant drug). The acetyl derivative gave a comparably remarkable

antioxidant activity than Vitamin C. However, the antioxidant activity of (CR) (Ladimejol) could not be regressed in this study. The antibacterial and antifungal activities elicited by both caffeic acid and obtained derivatives were concentration-dependent. Furthermore, the acetyl derivative (CA) was distinctly more bacteriostatic than the other two synthesized products against the test bacteria and was also hugely anti-candidal. In addition this derivative elicited higher activities than even caffeic acid. The reduced product (CR) (Ladimejol) recorded no growths in the screening for both antibacterial and antifungal activities.

Conclusion: The results from this study indicate that the synthesized novel aryl substituted alkenol, 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol) (CR) (Ladimejol) did not show any potentialities for antioxidant, antibacterial and antifungal activities. However, the acetyl caffeate (CA) demonstrated significant antioxidant, antibacterial and antifungal activities. Hence, acetyl caffeate could be considered as a lead candidate compound in the search for newer and more efficacious antioxidant and antimicrobial agents in more expanded structural activity relationship studies (SARS), drug design development and formulation studies.

Introduction

Over time, the harmful effects of reactive oxygen species (ROS) and free radicals (FR) have drawn increasing attention from man and the scientific world because the debilitations they cause in cellular injury and the aging process [1,2]. These reactive oxygen species easily initiate the per-oxidation of membrane lipids leading to the accumulation of lipid peroxides in the body while under pathological conditions and an imbalance is created between these reactive oxygen species and antioxidant defense mechanism. This eventually leads to oxidative stress due to modification in cellular membrane [3]. In an attempt to remedy this malady, antioxidants are routinely employed. Caffeic acid is a naturally occurring plant-based poly-phenolic compound found in coffee, turmeric, thyme, oregano, cauliflower, mushrooms, vegetables and fruits such as berries, and pears [4].

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This acid can also be obtained via the Shikimic acid pathway [5], from *Escherichia coli* on tyrosine substrate and by the conversion of p-coumaric acid to caffeic acid by the fungal isolate *Pycnoporus cinnabarinus* [6]. Caffeic acid is a phenolic hydroxycinnamic acid. The trans isomer is more common and active and its IUPAC nomenclature is (E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid [7,8]. The hydroxyl groups in caffeic acid enable the donation of hydrogen atoms to the stabilization of the resultant phenoxyl radical. The unsaturated side chain's double bond (2, 3 double bond) further enhances the stability of the phenoxyl radical. Additionally, studies suggest that caffeic acid can form chelates with divalent metals further contributing to its multifaceted antioxidant capabilities [9][10]. These comprehensive explorations of caffeic acid underscore its intricate bio-functionalities and potential therapeutic applications making it a worthy candidate chemical or drug for more investigations. Other biological applications of caffeic acid are found in anti-diabetic therapy [11], anti-inflammation [12,13], anti-carcinogenicity [14], cardio-protection [15], antithrombotic therapy [16] and anti-aging properties [17]. The obvious and incontrovertible gamut of the multifaceted uses of this acid necessitated the present

research. Consequently, this research was designed firstly to chemically modify caffeic acid via esterification, acetylation and reduction reactions. Secondly, it was also envisaged that new and potentially more active drug templates could result from this study. Furthermore, the acid and the obtained derivatives were to be screened for antioxidant activity (IC_{50}) using the rapid bench-top bioassay employing the DPPH reagent while the agar-diffusion method was to be used in evaluating the anti-bacterial and antifungal sensitivity properties employing the use of typed clinical isolates of gram (+) (*Staphylococcus aureus*), gram (-) (*E. coli)* bacteria and a fungal strain *(Candida albicans*). In addition, comparison of results obtained will be done with values obtained with acid, the chemically modified derivatives, Vitamin C (antioxidant drug), chloramphenicol (antibiotic) and fluconazole (antifungal drug) all clinical positive controls with a view to determining if any improvements could be observed in the biological activities of the synthesized compounds.

Experimental

Reagents/solvents

The following reagents and solvents were so obtained *viz* DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate**)** was purchased from Tianjin Kernel Chemical Reagent Company, China, Caffeic acid from Kamel Chemical Company, China and vitamin C was obtained from Fidson Pharmaceuticals, Nigeria respectively. Solvents and reagents namely, acetic acid, acetic anhydride, di-ethyl ether, ethanol, ethyl acetate, hydrochloric acid, iodine, magnesium sulphate, methanol, n-hexane, liquid paraffin, petroleum-ether, pyridine HCl, sodium borohydride, sodium hydroxide, sulphuric acid and tetrahydrofuran were sourced as AnaLAR Grade Chemicals from the British Drug House Chemicals Limited, Poole, England.

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Solubility tests for caffeic acid

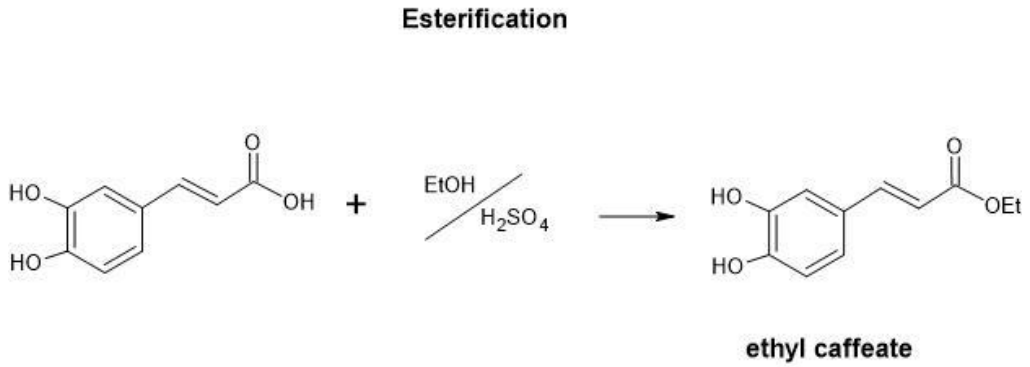
The solubility profiles of caffeic acid were determined by adding separately between five (5) and eight (8) mL of different solvents namely, acetic acid, ethanol, ethyl acetate, dilute hydrochloric acid, dilute sulphuric acid, methanol, n-hexane, petroleum ether, dilute hydrochloric acid, dilute sulphuric acid and water (hot) to caffeic acid (0.05 g) in different test tubes and observation was made for solubility (dissolution) or otherwise.

Determination of melting point of caffeic acid

A small quantity of caffeic acid (0.02 g) was carefully transferred to capillary tube whose one end has been previously sealed by a laboratory gas flame. The capillary tube was attached to an electro-thermal thermometer using a thin wire thread. A beaker (50 mL) containing quantity of liquid paraffin (25 mL) was placed on a wire gauze (mesh) on a tripod stand. The thermometer with the attached capillary tube was clamped onto a retort stand and immersed in the liquid paraffin bath. The beaker was gradually heated with constant stirring to ensure uniformity of temperature throughout the bath. The temperature at which the caffeic acid started melting and the temperature at which the acid completely melted were recorded and the melting point was subsequently obtained.

Synthesis of ester product of caffeic acid

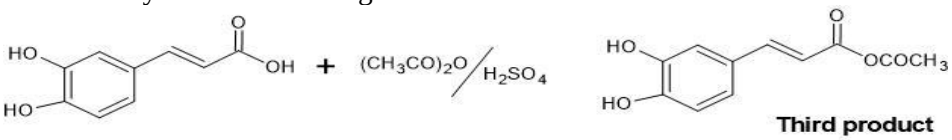
Caffeic acid (0.65 g) was weighed into a conical flask (250 mL). Ethanol (50 mL) was added, and the mixture was gently stirred with a glass rod until the particles were completely dissolved to obtain a clear solution. Another ethanol (50 mL) was measured and added to the solution in the flask to ensure complete dissolution of the particles. Concentrated sulphuric acid (5 mL) was added to the solution to serve as a catalyst. The flask containing the solution was corked with aluminum foil to prevent air from passing through. It was left for two (2) weeks to ensure that a complete synthetic reaction was affected in the refrigerator at a temperature of between -3°C and 5°C



C_E = Ethyl caffeate

Synthesis of acetyl derivative

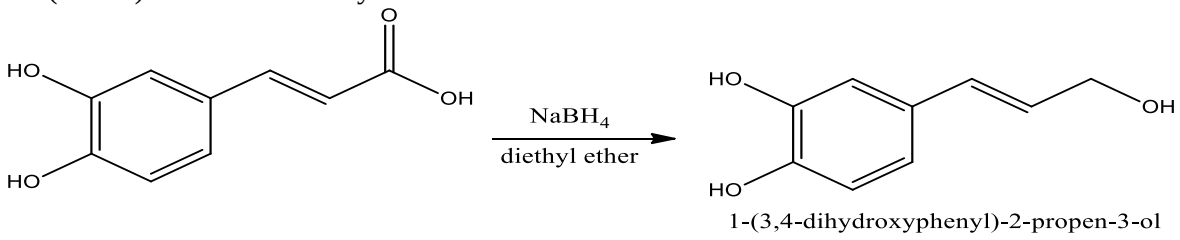
These methods were employed with some modifications. Caffeic acid (0.60 g) was dissolved in a mixture of acetic anhydride (15 mL) and acetic acid (15 mL) in a beaker. The solution was heated for thirty (30) minutes and allowed to cool. Concentrated sulphuric acid (7 mL) was added as catalyst. Further heating was done for a few more minutes. The mixture was covered with aluminum foil and kept in the refrigerator at a temperature of between -4°C and 5°C. After two (2) weeks, a yellow liquid was formed in the beaker. A moderately warmed diethyl ether (7 mL) was added, and the mixture warmed again for some minutes and allowed to cool before further use [18,19].



C_A = Acetyl caffeate

Synthesis of the reduction product of caffeic acid

This method was adopted with some modifications**.** A solution of caffeic acid (1.80 g) in diethyl ether was slowly added to a suspension of sodium borohydride (0.60 g) in diethyl ether (220 mL) at room temperature and left for fifteen (15) minutes. The mixture was then stirred until evolution of gas ceased. Iodine (0.65 g) and diethyl ether (25 mL) were added slowly to the mixture at a temperature of 0°C (in ice) with further evolution of hydrogen gas. The contents were further stirred for an hour and half. Dilute hydrochloric acid (8 mL) was added carefully and the mixture extracted with diethyl ether. The combined ethereal extract was washed with 3M sodium hydroxide (37 mL), brine and dried over silica gel. Evaporation of the organic layer gave the reduced product [20].



C_R = 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol (Ladimejol)

Determination of specific optical rotation of caffeic acid and its derivatives

The quartz tube in the polarimeter (ADP-220, Bellingham Stanley, England) was filled with distilled water and the machine subsequently zeroed. It was then emptied and refilled with a small quantity of caffeic acid solution (3 mL) in water and the value recorded. Similarly, the procedure was separately repeated for derivatives in their liquid states and likewise their values were recorded.

Determination of refractive index of caffeic acid and its derivatives

The refractometer (WAY-15, Abbe, England) is operated at the wavelength (λ) of sodium D line (589.3 nm) at 20.5ºC. The machine was switched on and allowed for fifteen (15) minutes to attain equilibrium. The refractive prism assembly was opened, and the mirror was removed. A syringe was used to deliver a clear aqueous solution of caffeic acid (4 m L) onto the prism. The prism assembly was closed, and its refractive index taken. This procedure was separately repeated for the three derivatives.

Infra-red spectroscopy of caffeic acid and derivatives

Caffeic acid (0.25 g) or 2.5 mL (liquid derivatives) each was analyzed for IR characteristics using the FTIR 84005 Spectrophotometer (Shimadzu, Japan).

Ultra-violet/visible spectroscopy of samples

Caffeic acid (0.2 g) dissolved in methanol or 2 mL of liquid derivative was analyzed for UV/VS absorption characteristics using the Jenway 6405 UV/VS Spectrophotometer.

Antioxidant activity

Spectrophotometric determination of antioxidant activity using DPPH reagent

The determination of antioxidant activity of a substance is premised on donation and acceptance of electrons by reacting chemical species. Hence, chemical reagents or species which are capable of donating electrons or hydrogen atoms can convert the purple-coloured DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-coloured non-radical form; 1, 1-diphenyl-2-picryl hydrazine [21,22]. Hence, the antioxidant activity of a compound can be evaluated by spectrophotometry.

Preparation of calibration curve for DPPH reagent

This experiment was carried out as described by both [23,24] with some modifications. DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions of the prepared stock solution were then carried out to obtain the following concentrations *viz,* 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036 % w/v. The absorbance of each of the sample was taken at λ_m 516 nm using the Ultra-Violet Spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken with a solution of methanol without DPPH which served as the blank.

Thin-layer chromatography of caffeic acid and derivatives

A portion of caffeic acid (0.04 g) dissolved in methanol (2 mL) or 2 mL of liquid samples (derivatives) was applied on a 15 cm x 10 cm silica gel analytical plate (Merck, Germany) and then developed in a toluene: acetone: water (10:20:1) mixture in a chromatographic tank until optimal separation and resolution was observed. The retardation factor (R_F) was then computed, thus:

$$\mathbf{R_F} = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}}$$

Determination of the antioxidant activity of caffeic acid, derivatives and Vitamin C

4 mg of sample was mixed with 100 mL of methanol. Serial dilutions were carried out to obtain the following concentrations: 0.0004 mg mL^{-1}, 0.0008 mg mL^{-1}, 0.0012 mg mL^{-1}, 0.0016 mg mL^{-1} and 0.0020 mg mL^{-1} using methanol. 6 mL of each concentration was incubated with 7 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of thirty (30) minutes in the dark at room temperature (25 ± 2^0C). An observation was then made for a change in the colour of the mixture from purple to yellow. The absorbance of each of the samples was taken at λ_m 516 nm. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus computed:

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

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 sample. Caffeic acid /synthesized derivative / Vitamin C concentration providing 50 % inhibition (IC_{50}) was calculated from a graph of inhibition percentage against the concentration of the caffeic acid/ synthesized derivative / Vitamin C [25-27]. Vitamin C was used as a standard antioxidant drug.

Antimicrobial Tests

The micro-organisms used in this study, namely, *Staphylococcus aureus* (NCTC 4598), *Escherichia coli* (NCTC 5463) and *Candida albicans* (NCYC 56) were

clinically isolated from specimens of diarrheal stool, urine, wounds and vaginal swabs obtained from the University of Uyo Health Centre, Uyo. The clinical isolates were collected in sterile bottles, identified and typed by convectional biochemical tests [28,29]. Preservation by refrigeration was done at 5^0C at the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy prior to further use. The hole-in-plate agar diffusion method was used observing standard operating procedures for bacteria and fungi respectively. The inoculum of each micro-organism was introduced into each petri-dish (Pyrex, England). Cylindrical plugs were removed from the agar plates by means of a sterile cork borer (Pyrex, England) to produce wells with diameter of approximately six (6.00) mm. The wells were equidistant from each other and the edge of the plate [30,31]. Concentrations of 10 mg mL-^{1} and 20 mg mL-^{1} of caffeic acid, 7.5 mg mL-^{1} and 15 mg mL-^{1} of derivatives were introduced into the wells. Also, different concentrations of 5 µg mL-^{1} chloramphenicol (Gemini Chemicals, Nigeria), 1mg mL-^{1} of Nystatin (Diamond Healthcare Chemicals, Nigeria) and aqueous methanol (1:1) were introduced into separate wells as positive and negative controls respectively [32-35]. The determinations were carried out in triplicates. The plates were left at room temperature for two (2) h to allow for diffusion. The plates were then incubated at 37 ± 2^0C for twenty-four (24) h. Zones of inhibition were measured in millimeters (mm).

Results

Table 1: Calibration curve for DPPH reagent at λ_{max} 516 nm

Concentration	Average absorbance (± 0.002)
0.0004	0.064
0.0008	0.166
0.0012	0.234
0.0016	0.328
0.002	0.39
0.0024	0.445
0.0028	0.538
0.0032	0.653
0.0036	0.708
Blank Absorbance of 0.004%w/v DPPH reagent: (0.869)	

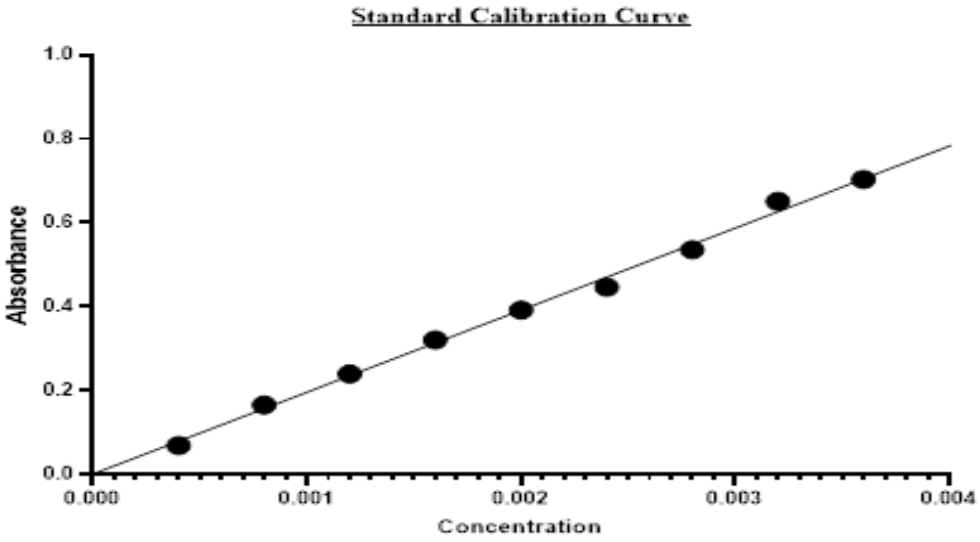


Figure 1: Graph of absorbance against concentration of DPPH reagent.

Table 2: Absorbance of samples incubated with DPPH at different concentrations at λ max 516 nm (Blank absorbance of 0.004% DPPH reagent: 0.869) (± 0.002).

Sample (mg mL^{-1})	0.0004	0.0008	0.0012	0.0016	0.002
Vitamin C	0.12	0.119	0.118	0.117	0.116
Caffeic acid	0.348	0.251	0,241	0.111	0.093
C_E	0.241	0.179	0.162	0.144	0.127
C_A	0.048	0.037	0.028	0.027	0.025
C_R	0.672	0.581	0.567	0.543	0.552

C_E = Ethyl caffeate; C_A == Acetyl caffeate; C_R = 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol (Ladimejol) -- reduced product; DPPH = 2, 2-Diphenyl-1-picryhydrazyl hydrate

Table 3: Radical scavenging activity (percentage inhibition %) of samples at different concentrations and IC50 of samples (±0.02).

Sample (mg mL^{-1})	0.0004	0.0008	0.0012	0.0016	0.002	IC_{50}(µgmL^{-1})
Vitamin C	56.19	86.31	86.42	86.54	86.35	0.18
Caffeic acid	59.95	71.12	72.27	87.23	89.3	0.22
C_E	72.27	79.4	81.46	83.43	85.39	0.19
C_A	94.48	95.74	96.78	96.89	97.12	0.16
C_R	22.67	33.14	34.75	36.48	37.51	NR

Refer to Table 2; RSA % (PI %) = Radical Scavenging Activity (Percentage Inhibition %); IC_{50} = Concentration at which 50% of DPPH is scavenged or inhibited; NR = Not regressed.

Table 4: Antibacterial screening of caffeic acid and its derivatives at different concentrations on test microbes in aqueous methanol (1:1) (± 0.01 mm)

Test microbe	Caffeic acid 10 mg/mL	Caffeic acid 20 mg/mL	C_E 7.5 mg/mL	C_E 15 mg/mL	C_A 7.5 mg/mL	C_A 15 mg/mL	C_R 7.5 mg/mL	C_R 15 mg/mL	Chloramphenicol 5 µg/mL	Me-OH/ H_2O (1:1)
S. aureus NCTC 4598)	18.00	25.00	NG	NG	38.00	46.00	NG	NG	48.00	6.00
E. coli (NCTC 5463)	19.00	30.00	NG	NG	32.00	40.00	NG	NG	43.00	6.00

The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +6.00) mm (Refer to Table 2). NCTC - National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK. ATCC- American Type Culture Collection, Washington, DC; NG- No Growth.

Table 5: Antifungal sensitivity screening of caffeic acid and synthesized derivatives at different concentrations on a fungal strain in Me-OH/H_2O (1:1) (± 0.01 mm)

Test microbe	Caffeic acid 10 mg/mL	Caffeic acid 20 mg/mL	C_E 7.5 mg/mL	C_E 15 mg/mL	C_A 7.5 mg/mL	C_A 15 mg/mL	C_R 7.5 mg/mL	C_R 15 mg/mL	Fluconazole 1 mg/mL	Me-OH/ H_2O (1:1)
C. albicans NCYC (56)	6.00	21.00	3.50	18.60	39.00	62.80	NG	NG	40.00	6.00

The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +6.00) mm (Refer to Table 2). NCYC- National Collection of Yeast Cultures, UK; NG--No Growth.

Caffeic acid: C_9 H_8 O_4 ; mol. wt. (180.16 g/mol); odourless brownish yellow crystalline solid; m.pt. (221-223 ^{0}C); $[n]_D^{20}$ (1.3934); $[\alpha]_D^{20}$ (+0.267 ^{0}); λ_{max} (230 nm); R_F (0.58); FTIR (cm^{-1}): 1589 (-Ar-C=C), 1614 (acyclic -C=C), 1723 (-C=O), 2885 (-CH stretching) 3130 (alcoholic –OH) and 3479 (-Ar-OH).

Ethyl caffeate (C_E): C_{11} H_{12} O_4; mol. wt. (208.21 g/mol); odourless colourless liquid; $[n]_D^{20}$ (1.467)6; $[\alpha]_D^{20}$ (+0.262 ^{0}), λ_{max} 326 nm); R_F (0.81); FTIR (cm^{-1}): 987 (alkyl bending mode, 1203 (-C-O-C, ether linkage), 1586 (-Ar-C=C), 1612 (acyclic -C=C), 1694 (-C=O), 2877 (-CH) and 3471(-Ar-OH).

Acetyl caffeate (C_A): C_{11} H_{10} O_5; mol. wt. (222.32 g/mol); pale yellow liquid; $[n]_D^{20}$ (1.4770); $[\alpha]_D^{20}$ (+0.386 ^{0}); λ_{max} (352 nm); R_F (0.84); FTIR (cm^{-1}): 1607 (-Ar-C=C), 1676 (acyclic -C=C), 1717 (-C=O in -$OCOCH_3$), 1736 (-C=O) and 3375 (-Ar-OH).

1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol) (Ladimejol (C_R): C_9 H_{10} O_3, mol. wt. (166.04 g/mol); viscous yellow liquid; $[n]_D^{20}$ (1.5191); $[\alpha]_D^{20}$ (0 ^{0}); λ_{max} (332 nm); R_F (0.88); FTIR (cm^{-1}): 1587 (-Ar-C=C), 1612 (acyclic -C=C), 2935 (-CH stretching) 3125 (alcoholic-OH) and 3429 (-Ar-OH).

Discussion

Spectroscopic analyses

Caffeic acid, an odourless brownish yellow substance is also described as tanned yellow compound. It is a hydroxy-cinnamic acid which is a major sub-group in phenolic compounds with the phenyl ring substituted by hydroxyl groups at positions C-3 and C-4. Some monographic studies were carried out to determine its identity, purity, integrity and suitability for the present research. It was observed to be soluble in acetic acid, ethanol, methanol and hot water. However, it was insoluble in ethyl acetate, diethyl ether, dilute HCl, dilute H_2 SO_4, n-hexane and petroleum ether. The determined values of melting point and refractive index both fall within stated limits in literature. The UV λ_{max} (246 nm) absorption is indicative of the presence of electron densities dispersed over -Ar-C=C, -Ar-OH, acyclic -C=C and alcoholic -OH chemical species while retardation factor R_F (0.58) shows that the acid is moderately polar and hence likewise retarded on the silica gel. The IR spectral matrix of caffeic acid shows stretching's at 1589, 1614, 1723, 2885, 3130 and 347 cm^{-1} which are diagnostically characteristic of - Ar-C=C, acyclic -C=C, -C=O, -CH bending modes, alcoholic -OH in carboxylic acid and -Ar-OH groups respectively. Ethyl caffeate (**C_E**), an aromatic phenol ester was synthesized as an odourless colourless liquid compound. The UV λ_{max} (326 nm) absorption which is comparatively higher than that of the pro-drug (caffeic acid) indicates the presence of electron clouds over -C-O-C, -Ar-C=C, acyclic -C=C, -C=O and -Ar-OH chromophores. The retardation factor R_F (0.69) indicates that the ester derivative is comparably non-polar and hence weakly retarded on the silica gel plate. Its IR spectrum shows peaks at 987, 1202, 1586, 1612, 1694, 2887 and 3471 cm^{-1} which are diagnostic of alkyl bending modes, hence accounting for the disappearance of the alcoholic OH peak at 3130,-C-O-C, -Ar-C=C, acyclic -C=C, -C=O, -CH and -Ar-OH respectively. It is instructive to note that the peak representing -C-O-C is diagnostically elucidative indicating that the hydrogen atom in the alcoholic -OH had been replaced with an ethyl group (-CH_2CH_3) showing that esterification of caffeic acid had been effected. Ethyl caffeate has been obtained via Co-A acylytransferase expression on *E. coli* [36] and is naturally occurring in diverse plants such as *Prunus yedensis*, *Polygonum amplexicale* and *Bidens piloso* from where it has been separately isolated [37]. Its radio-sensitizing effects have been studied and documented [38]. Furthermore, it is used as an anti-aging agent in cosmetology, treat inflammatory disorders and suppresses NF-Kappa B

activation. The acetyl product (C_A) was obtained as a pale yellow liquid. In addition, the UV λ_{max} (352 nm) absorption which is also higher than that of caffeic acid suggests that electrons are found delocalized over -Ar-C=C, acyclic -C=C, -$OCOCH_3$ and -Ar-OH chromophoric species. The retardation factor R_F (0.66) indicating some level of lipophilic character ensures it is comparably less hindered on the silica plate. The IR spectral matrix of the acetyl derivative is replete with peaks at 1607, 1676, 1717, 1736 and 3375 cm^{-1} accounting for -Ar-C=C, acyclic C=C, -C=O in $OCOCH_3$ -C=O and -Ar-OH chemical species. It is noteworthy that the hydrogen atom in the alcoholic -OH was replaced with the acetyl group in the acetylation process hence ensuring the disappearance of the alcohol peak at 3130 cm^{-1} inherent in the parent caffeic acid. The reduced product, 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol) (Ladimejol) (C_R) was synthesized as a highly viscous dark yellow liquid. The UV λ_{max} (332 nm) absorption is indicative of presence of electrons moieties in -Ar-C=C, acyclic -C=C, -OH and -Ar-OH chemical species while R_F (0.88) shows that this derivative is inherently non-polar because of the increased presence of non-polarity occasioned by the conversion of the -C=O to a -CH_2 thereby enhancing its relatively free movement on the silica-coated plate. The IR spectrum of the (C_R) is characterized by stretching's at 1587, 1612, 2933, 3125 and 3429 cm^{-1} which are indicative of the presence of -Ar-C=C, acyclic C=C, -CH, alcoholic -OH and -Ar-OH species. It noteworthy to highlight that the reduction reagent ($NaBH_4$ /I_2) selectively reduces the -C=O to -CH_2 [20] while the -C=C whether acyclic or aromatic is untouched. Surprisingly, in previous studies by this lead author and other co-workers, the three (3) endocyclic -Ar-C=C bonds were also reduced in vanillin and gallic acid [23] [24] because the -C=O specie was directly attached to the aromatic phenyl ring in the compounds. This translated to losses of the aromatic character in both vanillin and gallic acid respectively. However, if the -C=O is far from the aromatic ring (when not found attached to the aromatic ring), only the -C=O will be selectively reduced and not the -Ar-C=C bonds as observed in [20] and this present investigation. Comprehensive literature search of organic chemistry data libraries indicates that this reduced derivative (C_R) whose IUPAC nomenclature is 1-(3,4-dihydroxy phenyl)-2-propen-3-ol (3,4-benzenediol-2-propen-3-ol) is new and hereby given the trivial identity of Ladimejol. It was observed that caffeic acid, C_{E-}, C_A, and C_R showed optical rotation $[\alpha]_D^{20}$ of +0.267, +0.262, +0.386 and 0 ^{0} respectively indicating that the pro-drug and the derivatives are optically active except the reduced product. Hence, the reduced derivative, 1-(3,4-dihydroxy phenyl)-2-propen-3-ol) (3,4-benzenediol-2-propen-3-ol (Ladimejol) will demonstrate neither laevorotation (-) (ability of a compound to rotate plane of light in anticlockwise direction) nor dextro-rotation (+) (ability of a compound to rotate plane of light in clockwise direction). However, caffeic acid, ester and acetyl derivatives will elicit dextro-rotation (+) (ability of a compound to rotate plane of light in clockwise direction) [39,40]. This reduced derivative belongs to the class of compounds known as phenyl-propenoids which are related to catechols because of the attachment of the aryl component to an allyl alcohol.

Antioxidant activity

A calibration curve was prepared for DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) reagent with the aim of ascertaining its purity and suitability for use in the antioxidant evaluations. The Beer-Lambert's Law remains the basis of all absorption spectrophotometry [22]. The calibration curve obtained confirms that the underlying principles behind the Law were obeyed as the curve (Figure 1) shows a straight line which passed through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 516 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (caffeic acid /derivative/vitamin C) increased which suggested that the DPPH reagent was being reduced (Table 2). It was observed that as the radical scavengers were being introduced separately into the different beakers containing the DPPH reagent, a colour change from purple to yellow occurred for all except the reduced product (**C_R**) which gave a very dark brownish yellow colour probably because of its very viscous characteristics. Furthermore, Table 3 displays radical scavenging activity (RSA %) or percentage inhibition (PI %) and the computed IC_{50} values of caffeic acid /derivative / vitamin C. The RSA % is an indicator of the antioxidant activity of a compound. Interestingly, caffeic acid, ester derivative (C_E) and acetyl product (C_A) all demonstrated significantly remarkable antioxidant activity (IC_{50}) of 0.22, 0.19 and 0.16 µg mL^{-1} respectively. These values compare favourably with that of a standard antioxidant drug (Vitamin C) at 0.18 µg mL^{-1}. The ester and acetyl products both essentially contain chemical species such as -CH_2 CH_3 and -$COCH_3$ which confer some lipophilic character on them thereby enabling the compounds to get to the allosteric sites where the pharmacological action of anti-oxidation is to be effectuated in living organisms. Furthermore, the acetyl derivative gave the

most significant antioxidant activity (0.16 µg mL^{-1}) amongst all the compounds tested including being even more antioxidant than Vitamin C in this study. From the foregoing, it can be inferred that both esterification and acetylation separately enhance the antioxidant activity of caffeic acid. However, the activity of the reduced product could not be regressed in this present study. It is somewhat necessary to point out that the ester and acetyl derivatives of gallic acid and cinnamic acid also demonstrated equally remarkable antioxidant activities in previous studies by the lead author and co-workers [24,41].

Antibacterial tests

The microbes used in the sensitivity tests attested to the antibacterial spectrum encompassing one (1) gram positive bacterium namely, *S. aureus* (NCTC 4598) and one (1) gram negative bacterial species, E. *coli* (NCTC 5463). The results as presented in the Table 4 show that the antibacterial activity was concentration dependent. The higher the concentration of the antibacterial compound applied the higher the antibacterial activity. Caffeic acid and acetyl derivative were bacteriostatic at both concentrations (10 and 20 mg m L^{-1}) employed. Furthermore, the acetyl derivative (C_A) was more active than the pro-drug even at comparably lower concentrations (7.5 and 15 mg m L^{-1}) as can be seen in the Table 4. However, both the ester (C_E) and reduced (C_R) (Ladimejol) derivatives recorded no growths on the agar plates. The acetyl derivative was more suppressive of the *S. aureus* than *E. coli*. It is safe to imply that acetylation of caffeic acid enhances its antibacterial activity. Furthermore, it can be inferred from these results that acetyl derivative (C_A) could be a promising lead compound in the search for newer antibacterial agents for treatment and management infections of bacterial origins.

Antifungal screening

The antifungal screening was done with *C. albicans* (NCYC 56). Similarly, the antifungal activity demonstrated by caffeic acid and derivatives with the exception of the reduced derivative (C_R) (Ladimejol) are equally concentration dependent as displayed in Table 5. Furthermore, it was noticed that both caffeic acid and ester derivative (C_E) demonstrated some levels of anti-candidal activity but are somewhat inferior in comparison to the significantly remarkable activity demonstrated the acetyl derivative (C_A). The reduced derivative as observed in the antibacterial sensitivity tests also showed a no growth situation in the antifungal screening. It is probable that its viscous nature could be factor which this worker and fellow co-workers are interested in investigating in the subsequent stages of the studies on caffeic acid. However, it is convenient to infer that the acetyl derivative (C_A) may be a potential lead compound in the discovery of new antifungal drug templates especially in the treatment of candida-resistant infections.

Conclusion

This study reports for the first time the synthesis of 1-(3,4-dihydro phenyl)-2-peopen-3-ol (3,4-benzenediol-2-propen-3-ol) (**C_R),** a new aryl substituted alkenol from the reduction of caffeic acid's nucleus. It has been assigned the trivial name Ladimejol. This compound has demonstrated no antioxidant activity (IC_{50}), antibacterial and antifungal activities in this present study. Potential activities such as anti-inflammatory and anti-cancer amongst many others are to be worked on the reduced derivative in subsequent research. However, the acetyl derivative did demonstrate more excellent antioxidant, antibacterial and antifungal activities than the derivatives and even caffeic acid. It is noteworthy that the acetyl derivative (C_A) could be further studied in more expanded structural activity relationship studies (SARS), drug design development and formulation studies.

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Conflict of Interest

None declared.

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