ORIGINAL PAPER

https://doi.org/10.4314/njpr.v16i1.1

Nig. J. Pharm. Res. 2020, 16 (1) pp 1-8
ISSN 0189-8434 **e-ISSN 2635-3555** **Available online at http://www.nigjpharmres.com**

Synthesis, Characterization and evaluation of Anti-inflammatory and Antimicrobial Properties of some Cinnamic Acid Derivatives

B. S. JACOB[1BEF]; H. BABA[*2CDEF], J. O. OLUWADIYA[1ADEF]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Madonna University, Elele, Rivers state, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Cinnamic acid and derivatives are widely spread in plants. They are important intermediates in the synthesis of many aromatic compounds and are known to have diverse biological activities.

Objectives: To synthesize, characterize and evaluate some cinnamic acid derivatives, for possible antimicrobial and anti-inflammatory activities.

Material and methods: Different cinnamic acid derivatives were synthesized by Knoevenagel condensation reaction between malonic acid and various derivatives of benzaldehydes in the presence of pyridine and hydrochloric acid. The cinnamic acid epoxide was formed in the presence of hydrogen peroxide and KOH, while dihydro-cinnamic acid and paramethoxy-dihydro-cinnamic acids were formed by reducing cinnamic acid and paramthoxy-cinnamic acid respectively with raney nickel in the presence of 10% KOH. The final products were purified on column chromatography (CC), eluting with petroleum spirit/ethyl acetate. All the synthesized compounds were unequivocally characterized using the combination of UV-Vis Spectroscopy, infra-red (FTIR), ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy. In-vivo anti-inflammatory activities of the synthesized compounds was investigated using egg albumin-induced paw oedema in rat; while antimicrobial activity was evaluated by agar diffusion method.

Results: The compounds were obtained in high yield (30 – 97 %) and purity. 3,4-dioxomethylene cinnamic acid produced the highest (60.8%) and significant ($p < 0.05$) anti-inflammatory effect at the 2nd hour of the highest dose. Dihydro-cinnamic acid and cinnamic acid epoxide with 4mg/kg dose produced 55.5% and 54.9% inhibition of inflammation respectively at the second hour, while para-methoxy-cinnamic acid at the second hour with 2 mg/kg dose level produced 54.0% inhibition. The reference compound; diclofenac produced no significant inhibition of inflammation at 4mg/kg dose level. All the compounds showed mild antimicrobial activities against the tested organisms.

Conclusion: The findings from the study indicate that the synthesized compounds possess mild anti-inflammatory, with weak antibacterial and antifungal activities.

Keywords: anti-inflammatory, antimicrobial, cinnamic acid derivatives, Knoevenagel

INTRODUCTION

Cinnamic acid (3-phenylprop-2-enoic acid) and derivatives are very important intermediates in the biosynthesis of most aromatic natural products, being widely spread in plants and possessing wide range of activities (Christine *et al*.1984). Cinnamic acid derivatives had been reported to possess antioxidant, hepatoprotective, anxiolytic, insecticidal, antidiabetic, anticholesterolemic activities (Nam *et al*. 2001). Different substitutions on the basic moiety lead to various pharmacological activities with mild to moderate side effects which has made it difficult for the clinical application of these compounds. For instance, m- hydroxy or *p*- methoxy residue on cinnamic acid is reported to promote effective insulin releasing activity while 3, 4-dihydroxycinnamic acid (caffeic acid) has hepatoprotective activity (Nam *et al*. 2001).

Furthermore, cinnamic acids usually serve as starting materials for the synthesis of esters of commercial importance. Most Cinnamic esters of plant origin are useful in perfumery, cosmetic and pharmaceutical industries; for example, methyl caffeate shows antitumour and antimicrobial activities while methoxy substituted cinnamate like ethyl 3, 4, 5 trimethoxycinnamate , found in *Piper longum* plays a major role in controlling inflammatory diseases (Kumar *et al*. 2005).

Antimicrobial activity of cinnamic acid derivatives is as a result of the presence of ester and amide functional groups. Narasimhan and co-workers (2004) reported the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus, Bacillus subtilis;* antifungal activity against *Candida albicans* and *Aspergillus niger*. Isobutyl cinnamate and dibromo cinnamic acid showed strong antibacterial activity against Gram positive and Gram-negative bacteria in addition to good antifungal properties. The addition of halogens to the side chain caused remarkable increase in growth inhibitory effect of cinnamic acid whereas addition of hydroxy groups to the side chain double bond did not remarkably enhance the antimicrobial activity (Omura, 1976). The study by Neogi *et al.* 2003, clearly demonstrated that morroniside cinnamic acid conjugate has strong anti-inflammatory activity on E-selectin mediated cell–cell adhesion. For instance, 7-*O*-cinnamoylmorroniside exhibited excellent anti-inflammatory activity and was observed to be a potent inhibitor of TNF-α-induced E-selectin expression (Neogi *et al*., 2003). However, little is still known of the structural modifications of cinnamic acid derivatives which could contribute immensely to the development of new pharmacological molecules that can be utilized in the management of infections and control of inflammation. There is thus a need for more investigation on their side chain modifications and amination as well as structural elucidation of these groups of compounds. To this end, we have successfully synthesized four cinnamic acid derivatives by Knoevenagel condensation reaction and they were screened for anti-inflammatory and antimicrobial activity.

METHODOLOGY

Material

Benzaldehyde, malonic acid, cinnamic acid, pyridine, hydrochloric acid, anisaldehyde (*para*-methoxybenzaldehyde), ethanol, piperonal (3,4-dioxomethylenebenzaldehyde), p-dimethylaminobenzaldyde, sulphuric acid , raney nickel (AlNi.), hydrogen peroxide, sodium hydroxide, potassium hydroxide, methanol, acetic acid, petroleum spirit, ethyl acetate, diethylamine, dimethylsulfoxide, polysorbate 80 (Tween 80) were obtained from BDH chemical, England. Thin Layer Chromatography (TLC), Silica Gel 60 F 254 plates used to monitor the reaction, was obtained from Merck (Darmstadt, Germany).

Methods

Melting points were determined with an electrothermal melting point apparatus and were uncorrected. Infra-red (IR) spectra were measured on a Buck scientific IR M500 instrument. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 (250MHz), USA. Chemical shifts are reported in part per million (ppm) relative to tetramethylsilane (TMS). Mass spectra (MS) were recorded on a Varian MAT 44S mass spectrometer operating at 70eV

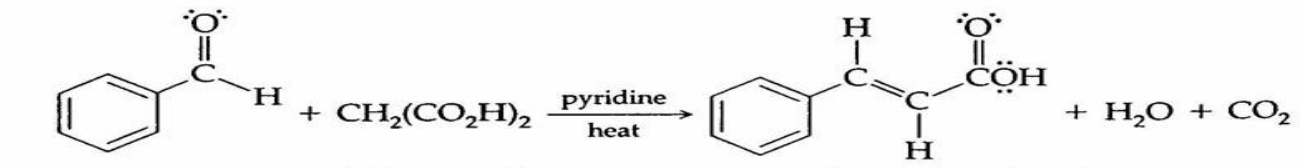


Figure 1: General reaction scheme

Methodology

Preparation of Para-methoxycinnamic acid

Anisaldehyde (p*ara*-methoxybenzaldehyde) (13.6 g) and malonic acid (10.4 g) were weighed, transferred into a flask; then 25 ml of pyridine was added to the mixture. The resulting mixture was then boiled under reflux for three hours. The mixture was poured into a beaker containing 40 ml of dilute HCl and about 15 ml of 12 M conc. HCl was added to acidify the mixture, a precipitate observed was allowed to settle and filtered. The product was recrystallized from hot ethanol. The crystals were collected, dried, weighed and the melting point determined.

Preparation of 3, 4-dioxomethylenecinnamic acid

Piperonal (3, 4-dioxomethylenebenzaldehyde) (15.0 g) and malonic acid (10.4 g) were weighed, transferred into a flask; 25 ml of pyridine was added into the flask. The mixture was then boiled under reflux for three hours. The mixture was poured into a beaker containing 40 ml of dilute HCl and about 15 ml of 12 M conc. HCl was added to acidify the mixture, a precipitate observed, was allowed to settle and filtered. The product was recrystallized from hot ethanol. The crystals collected, weighed, dried and the melting point determined.

Preparation of Dihydroxycinnamic acid

Cinnamic acid (4.5 g) was weighed and dissolved in 30 ml of methanol in a flask with gentle heating and constant stirring on a heating mantle. An aliquot of 10% KOH (30 ml) and raney nickel (5 g) were added to the solution; and allowed to stand for 30 minutes with constant stirring. The clear solution was decanted, and some amount of water was added to the raney nickel residue to prevent it from caking. The resulting solution was then acidified with 20 ml of 12 M conc. HCl, the precipitate observed was allowed to settle, filtered, dried and melting point determined.

Preparation of Paramethoxy-dihydroxycinnamic acid

Para-methoxy-cinnamic acid (1.70 g) was weighed and dissolved in 30 mL of methanol in a flask with gentle heating and constant stirring on a heating mantle. 30 ml of 10% KOH and raney nickel (5 g) were added to the solution. The solution was allowed to stand for 30 minutes with constant stirring after which the clear solution was decanted, and some amount of water was added to the Raney nickel residue to prevent it from caking. The clear solution was acidified with 20 ml of 12 conc. HCl the precipitate observed was allowed to settle, filtered, dried and melting point determined.

Preparation of Cinnamic acid epoxide

Cinnamic acid (1.48 g) was weighed and dissolved in 30 mL of methanol in a beaker; with gentle heating and constant stirring on a heating mantle. An aliquot of 10% KOH (10 ml) and H_2O_2 (15 ml) were added to the solution which was then allowed to stand for 30 minutes with constant stirring. The slightly foaming solution was acidified with 40 ml of 10% acetic acid, the precipitate formed was allowed to settle before it was filtered, dried and melting point determined.

Thin layer chromatographic determination

The precoated TLC plates used to monitor the reaction was developed in petroleumspirit/ethyl acetate (3:2) solvent system. The R_f are as follows: paramethoxycinnamic acid is 0.30; 3,4-dioxo-methylene cinnamic acid, 0.25; dihydrocinnamic acid, 0.35; paramethoxy-dihydrocinnamic acid, 0.32; cinnamic acid epoxide, 0.43.

Spectroscopic characterization

The mode of ionization for the mass spectrometry experiment is electron spray ionisation (ESI)

Animals

Wistar rats, 42 in number, (180 - 220 g) of either sex purchased from Niger Delta University animal house, Wilberforce Island, Nigeria were used. The animals were kept under the supervision of qualified personnel; with 12-hour light/ 12-hour dark cycles and were fed with grower feeds (Vita feed, Ibadan) and water *ad libitum*. Animals were fasted overnight, with free access to water prior to experiments. The study was carried out according to the "Principles of Laboratory Animal care"
(WHO 1985) and approved by the Institutional Animal Ethics Committee.

Anti-inflammatory study

Anti-inflammatory activity of the synthesized compounds was assayed using egg albumin-induced rat paw oedema assay model (Akah and Nnambie, 1994). The animals were divided into five groups (6 rats per group) of both sexes (excluding the pregnant females) and were orally administered a dose (1, 2 and 4 mg/kg) of the test compound per group. The positive control group received 4mg/kg of diclofenac while water was administered to the negative control group. After an hour egg albumin suspension (0.1 ml, 1 %) in normal saline solution was injected into the sub-plantar area of the right hind paw. The paw thickness was measured hourly over a period of 6

hour with the aid of veneer caliper. The same animals were used after a wash out period of two weeks to complete the animal experiment. Anti-inflammatory activity was evaluated and the percentage inhibition of oedema level by drugs was compared to control. Mathematically, anti-inflammatory was calculated as shown in equation 1 below:

Activity = %I = [ΔDc - ΔDt/ΔDc] x 100…….. (1)
Where ΔDt is the mean value for drug treated animals and ΔDc is the mean value for animals treated with water (negative control).

Micro-organisms
Micro-organism cultures (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albican*), were prepared aseptically with Muller Hinton Agar, sub-cultured and incubated at 37 ^{o}C for 24 hours. Muller Hinton agar (71.25 g) was dispersed in 1,250 ml of distilled water making one and half strength of Muller Hinton agar. The mixture was dissolved by gently heating on a hot water bath and 25 ml was measured into 50 McCartney bottles respectively. The bottles were all sterilized by autoclaving at 121 ^{o}C for 15 minutes and allowed to cool. In sub-culturing, three bottles each containing 25 mls of Muller Hinton agar out of the 50 prepared were aseptically poured into four different petri dishes after gentle shaking, allowed to set and labeled with the organism to be subcultured, (*E coli, S. aureus, P. aeruginosa and C. albican*, respectively*)*, a loop of each organism obtained was taken aseptically, streaked on the agar surface respectively, incubated at 37 ^{o}C for 24hours to observe growth. After 24 hours, one colony forming unit (cfu) of the sub-cultured isolates was suspended in 20 ml of sterile normal saline (0.9 % NaCl) and was compared with McFarland standard (5 % $BaSO_4$ solution) turbidity.

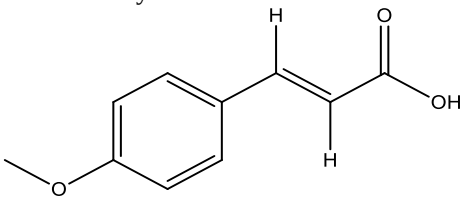
Antimicrobial assay
Thirty-three petri dishes were provided as determined by the number of microorganisms to be used and the number of pure compounds synthesized with the various concentrations. Each of the suspended organisms (0.5 ml) was pipetted and transferred aseptically into the petri dishes respectively. The bottles containing 25 mls of the molten agar at temperature between 40 – 45^{o}C were poured aseptically into each petri dish containing 0.5 ml suspension of the test organisms; and the plates were thoroughly mixed by swirling on the bench. On setting, wells were bored on the agar surfaces seeded with the test organisms using size 10 mm cork borer; four wells per plates, after which four different concentrations (10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1mg/ml), of the synthesized compounds were aseptically introduced into the wells. The plates were left undisturbed on the bench for 30 minutes to enable the compounds adsorb adequately. Ciprofloxacin and ketoconazole antimicrobial disc and powder were used as the standard drugs for antibacterial and anti-fungal assay respectively. The plates were then incubated at 37 ^{o}C for 24 hours after which they were observed for confluent growth of the microorganisms and clear zones of inhibition around the samples in the wells. The zones of inhibition were measured in millimeters (mm) across the centers of the wells. The average value of the readings for each well was taken as the zone of inhibition. The zone of inhibition was compared with the control group using the method of Bonev *et al*. (2008), and the percentage of inhibition is shown in table 2.

Statistical analysis
The results obtained were analyzed by Student's t-test and multiple comparisons were done by one way analysis of variance (ANOVA). A probaility level of less than 5% was considered significant ($p < 0.05$).

RESULTS AND DISCUSSION

Chemistry
The compounds were obtained in good yield (30 – 97 %) and high purity as shown by the melting point and TLC.

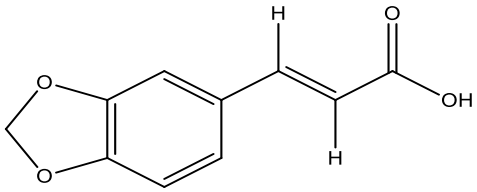
Para-methoxycinnamic acid



(2E)-3-(4-methoxyphenylprop-2-enoic acid

Yield: 6.237g (35.05 %), Melting point: 150 - 152 ^{o}C. UV-VIS (1 mg/ml), 280 nm (0.166). FTIR (KBr): 2941.54 (O-H), 1629.90 (C=O), 1242.20, 979.87). ^{1}HNMR (DMSO) δ ppm: 3.77 (s, 3H, OCH_3), 6.35 (d, J = 16 Hz, 1H, =CH), 6.94 (d, J = 8.5 Hz, 2H, Ar-H), 7.53 (d, J = 16 Hz, 1H, =CH), 7.61 (d, J = 8.5 Hz, 2H, Ar-H). ^{13}C NMR **(**DMSO) δ ppm: 55.7 (H_3CO), 114.8 (Ar-C), 116.9 (Ar-C), 127.3 (Ar-C) 130.4 (Ar-C), 144.2 (C=C), 161.38 (C=C), 168.27 (C=O).

3, 4-dioxomethylenecinnamic acid

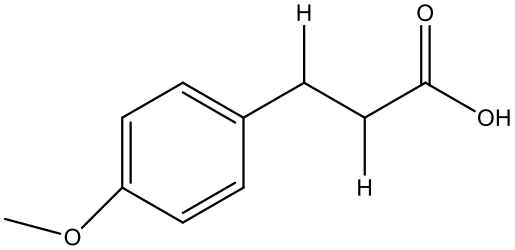


3, 4-dioxomethylenecinnamic acid

Yield 6.255g (32.58 %), Melting point: 228 - 230ºC.UV-VIS (1mg/ml), 318 nm (0.360). FTIR (KBr): 2939.61 (O-H) 1653.05 (C=O), 1460.16 (C-C), 1249.91 (C-O), 1024.24 (C=C), ^{1}HNMR (DMSO) δ ppm: 6.05 (s, 2H, H_2CO_2), 6.37 (d, J = 16 Hz, 1H, -C=C-H), 6.91(d, J = 8 Hz, 1H, Ar-H), 7.13 (d, J = 8 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-C), 7.45 (d, J = 16 Hz, 1H, -C=C-H). ^{13}C NMR **(**DMSO) δ ppm: 102.0 (H_2CO_2). 107.1 (Ar-C), 108.9 (Ar-C), 117.5 (Ar-C), 125.0 (Ar-C), 129.1 (-C=C), 144.4 (-C=C), 148.4 (Ar-C-O), 149.6 (Ar-C-O), 168.2 (C=O).

Dihydrocinnamic acid

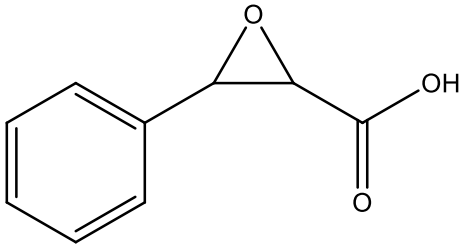
Paramethoxy-dihydrocinnamic acid



3-(4-Methoxyphenyl) propanoic acid

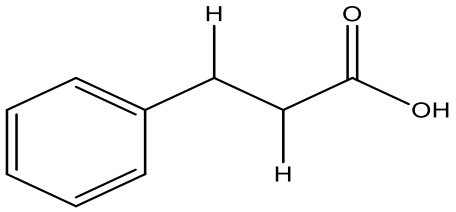
Yield 1.669 g (97.03 %), Melting point: 117120 ºC. UV-VIS (1mg/ml), 350nm (0.164). FTIR (KBr): 1635.69 (C=O), 1236.41 (C-O), 3443.05 (O-H). ^{1}HNMR **(**DMSO) δppm: 2.68, 2.89, 3.75, 6.81, 7.12,

Cinnamic acid epoxide



3-Phenyloxirane-2-carboxylic acid

Yield 0.922 g (56.22 %), Melting point: 118 – 120 ºC. UV-VIS (1mg/ml), 268 nm (0.680), 214 nm (0.583). FTIR (KBr): 2989.76, (O-H). 1651.12,



3-Phenylpropanoic acid

Yield 3.466 g (76.01 %), Melting point: 78 - 80 ºC. UV-VIS (1mg/ml), 268 nm (0.850). FTIR (KBr) : 3437.26 (O-H). 1653.05 (C=O), 1454.38 (C-C), 1255.70 (C-O). ^{1}H NMR (DMSO) δ ppm: 2.69, 2.94 (H-C-C-H), 4.458 (O-H), 7.49 (Ar-H). ^{13}C NMR **(**DMSO) δ ppm: 31(C-H), 36 (C-H), 126, (Ar-C) 128 (Ar-C), 140 (Ar-C), 179 (C=O).

9.23 ^{13}C NMR **(**DMSO) δ ppm: 55.74 (H_3-CO), 114.8 (Ar-C), 116.9 (H_2C-), 127.2 (Ar-C), 130.4 (Ar-C), 144.2 (H_2C-), 161.4 (Ar-C-O), 168.25 (C=O).

(C=O), 1296.21, (C-O), 939.36, (Ar-C=C). ^{1}HNMR **(**DMSO) δ ppm: 6.53 (d, J = 10 Hz, 1H, H-C-O), 7.41 (t, J = 2.5 Hz, 3H, Ar-H)7.54 (d, J = 10 Hz, 1H, H-C-O). 7.68 (q, J = 2.5, 2H, Ar-H). ^{13}C NMR **(**DMSO) δ ppm: 119.7 (Ar-C). 128.6 (Ar-C), 129.3 (Ar-C), 130.7 (Ar-C), 134.7 (-C-O), 144.4 (-C-O), 168.1 (C=O).

Anti-inflammatory effect of cinnamic acid derivatives

The *in- vivo* anti-inflammatory activity of the compounds was carried out using egg albumin-induced oedema assay, which is a working model of inflammation in the search for new anti-inflammatory agents that could be possibly used in therapeutics (Duffy *et al.* 2001). The oedema which develops in

rat paw after the injection of egg albumin in the sub-planar area is a biphasic event (Manueli *et al*. 1994). The initial phase is attributed to the release of histamine and serotonin; while the second phase (from the third hour) is attributed to prostaglandin, bradykinin, protease and lysosome (Vinegar *et al*.1969). The result in table 1 shows that, all the derivatives of cinnamic acid demonstrate significant ($p < 0.05$) anti-inflammatory activity at the second hour and mostly at the higher dose except paro methoxy cinnamic acid, which showed a significant inhibition of inflammation at 2 mg/kg dose level also at second hour. This indicates that the compounds mainly inhibit the first phase of the inflammatory process. Of the four compounds tested for anti-inflammatory activity, 3,4-dioxomethylene cinnamic acid sowed the highest activity (60.8%). It could also be observed, from table 1, that the 3,4-dioxomethylene cinnamic acid showed activity at the third hour, however, with 2mg/kg. This establishes the fact of its potency as the effective compound against inflammation.

Table 1. Percentage inhibition (%I) of cinnamic acid derivatives on egg albumin-induced paw oedem.
%I = [ΔDc - ΔDt/ΔDc] x 100

Time(hr) Drug	Dose (mg/kg)	1hr	2hr	3hr	4hr	5hr	6hr
H_2O	-	-	-	-	-	-	-
DCF	4mg	12.4	8.3	18.2	31.5	36.9	16.5
Para-methoxycinnamic	4mg	9.4	37.4	5.2	16.5	24.3	33.7
acid	2mg	9.9	54.0*	11.3	2.8	10.8	20.3
	1mg	32.0	32.1	15.8	17.3	30.2	47.5
3,4-dioxomethylene	4mg	17.8	60.8*	40.2	19.4	53.4	38.7
Cinnamic acid	2mg	7.4	14.5	53.3*	44.4	14.2	23.0
	1mg	9.6	45.7	19.2	26.2	1.5	8.1
Dihydrocinnamic acid	4mg	39.1	55.5*	16.8	5.2	12.1	29.9
	2mg	6.4	22.0	40.6	11.3	10.8	3.8
	1mg	19.3	11.6	15.1	0.0	18.3	5.4
Cinnamic acid epoxide	4mg	36.8	54.9*	7.6	15.7	17.9	10.0
	2mg	35.0	28.5	8.3	0.8	27.6	56.7*
	1mg	7.1	2.7	19.9	13.7	3.0	12.3

ΔDc = change in control, ΔDt = change in treatment, H_2O = Distilled water, DCF = Diclofenac $^*p < 0.05$, compared with control; paired t-test (n= 6)

This indicates that, formation of epoxide increases the anti-inflammatory properties of these compounds. Generally, it was observed that the activity of the compounds reduced after the second hour. This shows the compounds are not active at the late phases of inflammation and such may not be useful for chronic inflammatory conditions. However, it is interesting to note that, para-methoxy-cinnamic acid at the lowest dose (1mg/kg) showed remarkable inhibition of inflammation at the first and second hours but the higher doses (2 mg and 4 mg/kg) had no activity at those early hours. It was noticed also that the activity of the said compound at 1mg/kg declined after the second hour only to pick-up again at the fifth hour to produce its highest at the sixth hour. This unusual trend is common with compounds that act at different receptor sites. Though the overall result does not show any particular pattern of activity, the individual compounds can be said to have moderate anti-inflammatory activity. In the search for novel pharmacologically active compounds (antioxidant and anti-infectives), Cinnamic acid derivatives are important and promising compounds especially those with phenolic hydroxyl group (DiRosa and Willoughby 1971, Sova 2012). This is also applicable to their antimicrobial activities as shown in table 2, where the cinnamic acid epoxide shows some activity against *E. coli and C. albican.*

Table 2. Microbial activities of Cinnamic acid derivatives

Compound	Concentration	Zone of inhibition (mm)			
		P. aeruginosa	*S. aureus*	*E. coli*	*C. albicans*
Paramethoxycinnamic acid	10mg/ml	13	-	-	-
	5mg/ml	-	-	-	-
Dihydrocinnamic acid	10mg/ml	-	-	-	14
	5mg/ml	-	-	-	-
Paramethoxydihydrocinnamic acid	10mg/ml	-	13	-	16
	5mg/ml	-	-	-	-
Cinnamic acid epoxide	10mg/ml	-	-	15	15
	5mg/ml	-	-	-	-
CPX	10mcg	38	24	41	-
KTZ	5mg/ml	-	-	-	30

CPX=ciprofloxacin, KTZ=ketoconazole

CONCLUSION

The findings of the study demonstrate that, the synthesized compounds; cinnamic acid derivatives, though showing poor antimicrobial activity, have promising anti-inflammatory activity on further modifications on their basic moiety. The anti-inflammatory activity shown by the epoxide derivative is comparable to the reference drug (diclofenac). Thus, there is a need to further evaluate these compounds for their ant-inflammatory and/or possible analgesic activity.

REFERENCES

Akah, P.A. and Nnambie, A.I. (1994) Evaluation of Nigeria traditional medicine; Plants used for rheumatic (inflammation) disorders, J. Ethnopharmacol. 42: 179-182.

Bonev, B., Hooper, J., Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method, J. Antimic. Chemother. 61(6): 1295 – 1301.

Christine, S.V., Rohan, K.G., Ian B.R. (1984). The effect of food preservatives on pH homeostasis in *Escherichia coli, Journal of General. Microbiology*. 130 (11): 2845 - 2850.

DiRosa, M., Willoughby, D.A.(1971). Screens for anti-inflammatory drugs, J. Pharmacol. 23: 297 – 298.

Duffy, J.C., Dearden, J.C., Rostron, C. (2001). Design, synthesis and biological testing of a novel series of anti-inflammatory drugs, J. Pharm Pharmacol. 53:1505 – 1514.

Kumar, S., Arya, P., Mukherjee, C., Singh, B.K., Singh, N., Prasad, V.S., Ghose, A.K. (2005). Novel Aromatic ester from *Piper longum* and its analogues inhibit expression of cell Adhesion molecules on Endothelial cells, *Biochemistry*. 44: 15944-15952.

Manueli, V.E.F., Diaz, G., Gonzalex, A., Bermejo, J. (1994). Antinociceptive, Anti-inflammatory and Antipyretic effects of Lapidin, a bicyclic sesquiterpene, Planta Medica. 60:395-399.

Nam, N.H., You, Y.J., Kim, Y.D., Hong, H., Kim, H.M., Ann, Y.Z. (2001). Synthesis of certain 3-Aryl-2-propenoates and evaluation of their cytotoxicity, *Bioorg. Med. Chem. Lett.* 11(9):1173.

Narasimhan, B., Belsare, D., Pharande, D., Mourya, V., Dhake, A.(2004). Esters, amides and substituted derivatives of cinnamic acid: synthesis, antimicrobial activity and QSAR investigations. *European Journal of Medicinal Chemistry*, **,** 39, 827–834

Neogi, P., Lakner, F.J., Medicherla, S., Cheng, J., Dey, D., Gowri, M., Nag, B., Sharma, S.D., Pickford, L.B., Gross, C. (2003). Synthesis and structure-activity relationship studies of Cinnamic acid-based novel thiozolidinedione antihyperglycemic agents, *Bioorganic & Medicinal Chemistry*. 11: 4059-4067.

Omura, S. (1976). The antibiotic cerulemin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis, *Bacteriol. Rev.* 40: 681-697.

Sova, M. (2012). Antioxidant and Antimicrobial activities of Cinnamic acid derivatives, Mini Rev. Med. Chem. 12(8): 749-762

Vinegar, R., Schreiber, W., Hugo, R. (1969). Biphasic development of carrageenan oedema in rats, J. Pharmacol. Exp. Ther. 166: 96-100.
World Health Organization (1985). Principles of laboratory animal care, Chronicles. 39: 51-56.

*Address for correspondence: Haruna Baba
[2]Department of Pharmaceutical Chemistry,
Faculty of Pharmacy,
Madonna University, Elele,
Rivers state, Nigeria
Telephone: +234-703-440-2435
E-mails: babharun1@gmail.com

Conflict of Interest: None declared
Received: January 11, 2019
Accepted: January 17, 2020