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Jatropha tanjorensis a Flora of Southeast Nigeria: Isolation and Characterization of Naringenin and Validation of Bio-enhanced Synergistical Activity of α-Tocopherol Toward Clinical Isolates of Resistant Bacterial

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Abstract

Jatropha tanjorensis is among the rich floras of Southeast Nigeria and used by ethnic people to treat infections and manage health conditions. Ethnomedicine has long been employed in the treatment of ailments caused by bacterial pathogens. Studies showed that the incorporation of synthetic α-tocopherol to an antibacterial agent improves its activity. However, knowledge about the antibacterial-enhanced activity of plant-based α-tocopherols, especially those isolated from *Jatropha tanjorensis*, is limited because of the different bioactivities of synthetic and natural α-tocopherols. To determine the phytochemicals in *J. tanjorensis*, we carried out the structural elucidation of its leaf extracts. Naringenin and α-tocopherol were identified based on nuclear magnetic resonance experiments. To validate the bio-enhanced activity of α-tocopherol, we profiled the crude extracts, naringenin, α-tocopherol, and 1:1 mixture of naringenin and α-tocopherol against the resistant strains of local clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Streptococcus pneumoniae*, and *Staphylococcus aureus* using the average diameter of the zone of inhibition (AVDZI), minimum inhibitory concentration, and minimum bactericidal concentration. The results showed that the crude leaf extract ($\geq 10.2 \pm 0.17$ mm) and naringenin ($\geq 8.1 \pm 0.13$ mm) exhibited viable antibacterial activities, but α-tocopherol ($\leq 2.23 \pm 0.12$ mm) did not show any activity against all the bacteria assayed. In addition, the 1:1 mixture of naringenin and α-tocopherol ($\geq 8.7 \pm 0.13$ mm) showed an improved AVDZI, which suggested the bio-enhanced synergistical activity of its crude extracts induced by α-tocopherol. The results of these research validate the claims by ethnomedicine practitioners on the efficacy of *J. tanjorensis* in the treatment of infections.

Keywords: antibacterial, ethnomedicine, J. tanjorensis, naringenin, α-tocopherol

Introduction

Owing to the increased resistance of bacterial organisms toward synthetic drugs [1], other alternatives that pose less risk and maximum benefits must be explored and exploited. Ethnomedicine has been beneficial in the treatment of infections caused by bacterial pathogens; among its utmost benefits are its availability and ease of preparation, hence serving the ever-growing population of underdeveloped nations. Bacteria exhibit multifactorial resistance to synthetic drugs [1]; the misuse and overdose of drugs are among the major contributing factors. Southeast Nigeria is populated by ethnic Igbos who are known for their ethnomedicinal prowess; *J. tanjorensis* has long been used by "Dibia's" (native doctors/herbalist) in the treatment of various diseases and infections [2]. In Ebonyi State, Southeast Nigeria, *J. tanjorensis* is known locally as "ulo okwu di anya" (hospital too far) because it is believed to improve the hematological parameters of patients in need of urgent blood transfusion, including pregnant women and sickle-cell patients [3]. Previous research has shown that α-tocopherol and its esters manifest activity against bacteria in vitro; however, the pharmacodynamics of α-tocopherols differ [4]. Similarly, the enhancement of antibiotic activity by α-tocopherol has been studied [5]. *Jatropha* belongs to the Euphorbiaceae family, which includes over 170 species [6]. Over time, *Jatropha* species have been used as ethnomedicine by native people in tropical and subtropical countries [6]. The phytomedicine profiling of *Jatropha* species suggest that many compounds of medicinal relevance have been isolated; these compounds, which show cytotoxic activity, include curcusone A-J, 4-*epi*-curcusone E, and jatrocurcasenone from *J. curcus* [7]. Similarly, the cytotoxic activity of jatrogrossidion, 2-*epi*-hydroxyisojatrogossidion, 2-hydroxyisojatrogossidion, and 2-*epi*-isojatrogossidion from *J. grossidentata* has been reported [8]. The antimalaria and gastroprotective effects of jatropholone A-B from *J. dioica, J. isabellei*, and *J. gossypiifolia* have

been studied [9, 10]. Jatromulone A and jatromulodione A from *J. multifida* possess thioredoxin reductase inhibitory activity [11]. Similarly, the cytotoxicity of 6-*epi*-rhioloxatrione from *J. dioica* has been revealed [10, 12]. The toxicity and antimicrobial activity of *J. dioica* have been validated [13]. Ribofolin from *J. rhifolia* possesses antiplasmodial activity [14]. The crude extract of *J. curcus* showed antimicrobial activity, and the aqueous extract of *J. gossipiifolia* serves as an antivenom and shows an anticariogenic activity [15, 16, 17, 18]. *J. mollisima* has been reported to possess an antiphyretic potential [19], and *J. multifida* has antibacterial, anti-inflammatory, and antioxidant activities [20]. *J. neupeuciflora* exhibits antibacterial, antifungal, and antimicrobial activities and a wound healing effect [21]. Meanwhile, *J. gossypiifolia* has shown in vitro trematocidal activity [22]. In addition, *J. curcus* extracts possess antioxidant and larvicidal potentials [23, 24, 25]. Therefore, as *J. tanjorensis* contains a library of compounds [26] that possesses medicinal benefits [27] but remain to be fully harnessed for the treatment of infectious diseases, *Jatropha* species possess a broad spectrum bioactivity. Hence, this study aimed to provide valid data that will prove the ethnomedicinal efficacy of *J. tanjorensis*, which is used by ethnic Igbos of Southeast Nigeria to treat and manage infectious diseases, and correlate the *J. tanjorensis*-improved bioactivity to the presence of α-tocopherol.

Materials and Methods

J. tanjorensis leaves were collected from Echeaba in Ebonyi, Local Government Area of Ebonyi State in December 2020. The leaves were authenticated as *Jatropha tanjorensis* by a taxonomist at the Department of Applied Biology, Ebonyi State University [28]. The leaves were air dried and pulverized with the aid of a mechanical grinder (Corona, Nigeria) and a locally made mortar and kept for further use in an air-tight container. All chemicals used were of Sigma-Aldrich quality grade, and the melting point was determined using a Thiele apparatus (Duran, Germany). All the bacteria used were local clinical isolates, and the ampicillin used was Cikacillin® (Ampicillin Trihydrate BP 250 mg). Statistical analysis was conducted using SPSS version 20.

Extraction and purification. Sequential extraction of the plant part was successively carried out separately with solvents of increasing polarity: n-hexane, chloroform, methanol, and MeOH-H_2O mixture (4:1). A total of 10 kg pulverized leaves of each plant part were weighed and soaked in appropriate solvents in the order of increasing polarity for 72 h. The mixture was filtered, and the filtrate was concentrated using a rotary evaporator (Stuart RE 300/MS, UK) to one-tenth of its volume at < 40 °C [29] to prevent prolonged heating, which will cause oxidation and degradation of the desired extracts. Each dried extract was weighed in an analytical balance (OHAUS PX225D, USA) and stored at -4 °C.

Column and flash chromatographic separation. In each of methanol, n-hexane, chloroform and methanol/ water crude extracts, 15 g was subjected to column chromatography and eluted with n-hexane/ethyl acetate (80:20, 70:30, 60:40, and 50:50.), ethyl acetate (100%), and methanol (100%) gradients. The leaf extracts (15 g) were dissolved in the eluting solvent and packed on top of the silica gel slurry with a pipette. Then, the eluting solvent was added. Collection of the eluent was performed with 50 and 100 mL conical flasks. Further elution was conducted with increasing concentration gradients. For the methanol leaf crude extract, elution was carried out using dichloromethane/ethyl acetate (80:20 and 70:30), ethyl acetate (100%), ethyl acetate/ methanol (50:50), and MeOH (100%) gradients. For n-hexane leaf crude extract, elution was achieved with n-hexane/dichloromethane gradients (60:40 and 50:50), ethyl acetate (100%), ethyl acetate/methanol (50:50), and methanol/water (80:20). For chloroform leaf crude extract, elution was performed with n-hexane/ dichloromethane gradients (60:40 and 50:50), ethyl acetate (100%), ethyl acetate/methanol (50:50), and 100% methanol. The elution of methanol/water leaf extract was carried out with dichloromethane/ethyl acetate (80:20), ethyl acetate (100%), and methanol (100%). The extracts that gave the yield of analytical significance after column separation were used for further analysis. The fractions from methanol extracts were labeled JTR1 and JTR3. The fractions collected were monitored for purity by dissolving in methanol and spotting on thin-layer chromatographic plates using methanol/water (80:20) as the eluent. Further purification was carried out using a flash chromatographic technique in a solvent system of petroleum ether/ chloroform. The fractions were concentrated, dried, and washed several times with methanol, affording pure extracts labeled JTR1 and JTR3. Further, JTR3 was dissolved in methanol (50 mL) and recrystallized by heating in a rotary evaporator at 40 °C, followed by filtering of the hot solution. The filtrate was allowed to cool in a refrigerator (Haier Thermocool, UK) at 4 °C. Then, the crystals of JTR3 formed were filtered off using a filter paper (Whatman No. 42). For JTR1, a similar method was employed at a very low temperature range (-10 °C to -20 °C) [30]. The recrystallization was repeated thrice. The samples obtained were weighed and stored for use at 4 °C.

Structural elucidation. Prior to nuclear magnetic resonance (NMR) analysis. JTR1 and JTR3 were dissolved in deuterated chloroform and dimethyl sulfoxide-d_6, respectively. The ^{1}H, ^{13}C, DEPT-135, COSY, TOCSY, HMBC, and HSQC spectra of isolated compounds were acquired using an Avance III 500 MHz NMR spectrometer (Bruker, USA).

Evaluation of Antibacterial Activity

Determination of the diameter of zone of inhibition. Clinical isolates of the assayed bacteria were obtained from Alex Ekwueme Federal University Teaching Hospital Abakaliki. The agar well diffusion technique was used for antibacterial screening as described by CLSI [31], Daoud *et al.* [32], and Gonelimali *et al.* [33]. A total of 1 mL freshly prepared bacterial culture was pipetted at the center of a petri dish followed by pouring of Muller Hinton agar (MHA) into a petri dish containing the inoculum followed by thorough mixing. Then, the wells were made using a 6 mm-diameter sterile cork borer into agar plates containing the inoculums. Afterward, 1000 µL of each extract, isolated compounds, and control drug equivalent to a pre-determined concentration of 128% (w/v) were added to each well. Then, the plates were placed in a refrigerator for 1 h to allow the thorough diffusion of the extracts into the wells. Then, the plates were placed in an incubator (Eppendorf, Germany) for 24 h at 37 °C. The zones of inhibition were measured in millimeter, and the average of a triplicate measurement was calculated and recorded.

Determination of minimum inhibitory concentration (MIC). Concentrations of 64%, 32%, 16%, 8%, 4%, 2%, 1%, 0.5%, 0.25%, and 0.125% of crude extracts and isolated compounds from *J. tanjorensis* were prepared by serial dilution. The method described by Jorgensen and Ferraro [34] and Gonelimali *et al.* [33] was used for the analysis. Exactly 1 mL of each prepared inoculum was pipetted into a sterile petri dishes. Then, molten agar was added followed by thorough mixing. Four wells were made on each plate. Next, 1000 µL of 64%, 32%, 16%, 8%, 4%, 2%, 1%, 0.5%, 0.25%, and 0.125% of crude extracts and isolated compounds from *J. tanjorensis* were transferred to the respective wells. The plates were kept in the refrigerator for 1 h and incubated at 37 °C for 24 h. The lowest concentration that inhibited growth was considered as the MIC.

Determination of minimum bactericidal concentration (MBC). The paper discs in all the plates from MIC tests were reactivated using a mixture of 0.5% egg lecithin and 3% Tween 80 solution. The reactivated organisms were subcultured into appropriately labeled quadrants of the sterilized MHA plates. The organisms were uniformly streaked on labeled quadrants using a wire loop. The organisms were incubated at 37 °C for 24 h, after which the growth was observed and recorded. The determination of MBC was carried out by the method described by Jorgensen and Ferraro [34] and Adebayo *et al.* [35]. The MBC was the quadrant with the lowest concentration of the extract without growth.

Results and Discussion

Results of spectral elucidation. NMR spectra provide detailed information about the molecular structure and dynamics of phytochemicals. It is mostly used in natural product chemistry for structural elucidation and verification of molecules of plant origin. Both one-dimensional (1D) and 2D NMR analyses and spectral comparison with references were used to validate the structure of JTR1 and JTR3 as α-tocopherol (Figure 1) and naringenin (Figure 2), respectively.

***JTR1*: α-tocopherol.** *Yellowish-brown oil*; R_f 0.66; mass 27.87 g; yield 0.28 %; melting range 2 °C–3 °C; [36] ^{1}H-NMR (500 MHz, cdcl$_3$): δ_H 7.18 (1H, s, 6-OH), 2.38 (2H, t, H-4), 2.16 (3H, s, H-1'), 2.11 (3H, s, H-2'), 1.55 (3H, s, H-3'), 1.55 (3H, q, H-3), 1.49 (2H, m, H-11), 1.39–1.24 (4H, m, H-12, H-14, H-18), 1.23–1.20 (8H, m, H-13, H-15, H-16, H-20), 1.10 (3H, s, H-4'), 1.08–1.04 (7H, s, H-17, H-19, H-21, H-22), 0.76–0.73 (12H, t, H-5', H-6', H-7', H-23). ^{13}C (500 MHZ, CDCl$_3$): δ_C 145.5 (C-9), 145.5 (C-6), 122.6 (C-8), 121.0 (C-7), 118.5 (C-5), 117.4 (C-10), 74.5 (C-2), 39.9 (C-11), 39.4 (C-21), 37.5 (C-13), 37.4 (C-15), 37.3 (C-17), 32.8 (C-19), 32.7 (C-14), 31.5 (C-18), 31.5 (C-3), 30.0 (C-22), 30.0 (C-22), 24.8 (C-20), 23.8 (C-4'), 22.7 (C-7'), 22.6 (C-23), 20.8 (C-12), 19.7 (C-4), 19.7 (C-5'), 19.6 (C-6'), 12.2 (C-2'), 11.8 (C-3'), and11.3 (C-1'). Physical characterization and NMR spectra of JTR1 matched those of α-tocopherol referenced in literature [37, 38] and were thus assigned to Figure 1.

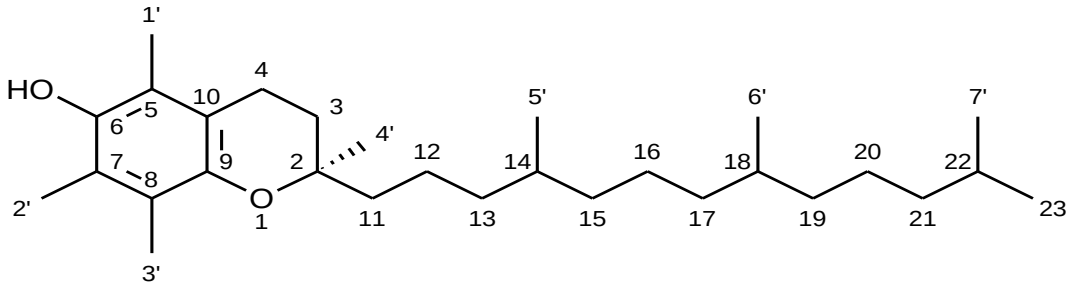

Figure 1. Structure of α-tocopherol ($C_{29}H_{50}O_2$) Isolated from *J. tanjorensis*

JTR 3: Naringenin. *White solid*; R_f 0.81; mass 9.93 g; yield 0.10%; melting range 253 °C–255 °C [39] ^{1}H (500 MHZ, dimethyl sulfoxide (DMSO)-d_6): 7.33 (2h, t, *J* = 7.15 Hz, H-6'), 7.30 (2H, d, H-2'), 6.83 (1H, s, H-5'), 6.80 (1H, s, H-3'), 5.90 (1H, s, H-6), 5.89 (1H, s, H-8), 5.30 (1H, s, H-2), α-3.10, β-2.69 (1H, dd, H-3). ^{13}C (500 MHZ, DMSO-d_6): δ_C 197.8 (C-4), 168.4 (C-9), 165.5 (C-5), 165.5 (C-7), 159.1 (C-4'), 131.1 (C-1'), 129.3 (C-6'), 129.1 (C-2'), 116.3 (C-3'), 116.3 (C-5'), 103.4 (C-10), 97.1 (C-6), 96.2 (C-8), 80.5 (C-2), and 42.1 (C-3). Physical characterization and NMR spectra of JTR3 matched those of naringenin reported in literature [39, 40, 41] and were thus assigned to Figure 2.

Results of antibacterial analysis. The AVDZI determines whether a bacterium is susceptible to specific antibacterial agents. Therefore, it is used to access the antimicrobial activity of a compound because it correlates the sensitivity of a bacterium to an antibacterial agent. The AVDZI was used to access the antibacterial activity of crude extracts and isolated compounds of *J. tanjorensis*. To test various levels of significance, we subjected the obtained results to Tukey's multiple comparison analysis using SPSS software version 20.

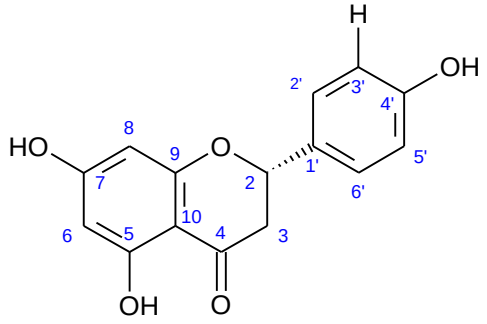

Figure 2. Structure of Naringenin ($c_{15}h_{12}o_5$) Isolated from *J. tanjorensis*

The experiment was performed in triplicate, and the results were expressed as mean ± standard error of the mean. Table 1 depicts the AVDZI of various extracts of *J. tanjorensis*. Ampicillin showed a better activity for all bacteria assayed, and the AVDZI for all extracts except JTR3 were comparable. Previous studies have reported the synergistic antimicrobial activity of JTR1 [42]. The antibacterial activities of JTR3 [43] and *Jatropha tanjorensis* [44, 45] have been profiled. Meanwhile, Oboh and Masodje [41] established the antimicrobial properties of *Jatropha tanjorensis*; Viswanathan *et al*. [45] attributed the broad spectrum antimicrobial activities of *Jatropha tanjorensis* to the presence of phytochemicals isolated from its methanol extract. Dada *et al*. [46] reported the antimicrobial activities of *Jatropha curcas*, indicating the wide antimicrobial application of *Jatropha* species in ethnomedicine. Andrade *et al*. [42] showed the improved antimicrobial activity and potentiation of aminoglycosides by α-tocopherol against multiresistant bacteria.

MIC is the lowest concentration of an antibacterial agent that inhibits the growth of a bacterium. MICs are mostly used to confirm the resistance and determine the in vitro antibacterial activity of novel antibacterial agents. The data obtained from MIC are used to propose MIC breakpoints. MIC was used to access the resistance and antibacterial activities of crude extracts and isolated compounds of *J. tanjorensis* in comparison with a known reference standard (ampicillin). From Table 2, MWCE gave the MICs of 1.000 and 0.250 mg/mL for *E. coli* and *K. pneumoniae*, respectively. CCE exhibited the MIC of 32.000 mg/mL for both *E. coli* and *S. typhi*.

JTR1 showed no MIC at the coverage concentration for all bacteria analyzed, and the MCE, HCE, and MWCE were 16.000 mg/mL for *S. typhi*. MCE showed MICs of 2.000, 2.000, 16.000, 4.000, and 0.250 mg/mL toward

Table 1. Average Diameter of the Zone of Inhibition (AVDZI) of *J. tanjorensis* Extracts *(mm)*

Antibacterial Agents	*Escherichia coli*	*Klebsiell pneumoniae*	*Salmonella typhi*	*Streptococcus pneumoniae*	*Staphylococcus aureus*
MeOH	-	-	-	-	-
MCE	13.0 ± 0.00^{a}	19.2 ± 0.19^{a}	11.2 ± 0.12^{a}	18.3 ± 0.20^{a}	17.2 ± 0.12^{a}
HCE	11.3 ± 0.21^{b}	20.3 ± 0.13^{a}	11.2 ± 0.15^{a}	15.0 ± 0.00^{b}	12.17 ± 0.09^{b}
CCE	10.3 ± 0.13^{c}	18.1 ± 0.06^{a}	10.4 ± 0.23^{c}	13.1 ± 0.09^{c}	15.3 ± 0.21^{c}
MWCE	16.1 ± 0.10^{d}	$20.0 \pm 0.00^{a, d*}$	10.2 ± 0.17^{c}	11.2 ± 0.12^{d}	16.3 ± 0.15^{d}
JTR1	2.0 ± 0.00^{e}	0.0 ± 0.00^{e}	0.0 ± 0.00^{e}	0.0 ± 0.00^{e}	2.23 ± 0.12^{e}
JTR3	9.0 ± 0.00^{f}	10.0 ± 0.00^{f}	9.2 ± 0.12^{f}	13.2 ± 0.17^{c}	8.1 ± 0.13^{f}
Ampicillin	19.1 ± 0.12^{g}	$23.0 \pm 0.09^{a, g*}$	17.2 ± 0.17^{g}	28.1 ± 0.12^{g}	25.2 ± 0.17^{g}

* "a, b, c, d, e, f, g" depict the various levels of significance between the antibacterial agents obtained from Tukey's posthoc analysis. Distinct letters in the same column indicate significance difference ($p < 0.05$), and similar letters in the same column shows non-significance difference ($p > 0.05$). Letters with * indicate that such groups are significantly different from each other but not significantly different from other antibacterial agents. - : no activity, MCE: methanol crude extract, HCE: hexane crude extract, CCE: chloroform crude extract, MWCE: methanol/water crude extract, JTR1: α-tocopherol; JTR3: naringenin.

E. coli, *K. pneumoniae*, *S. typhi*, *S. pneumoniae*, and *S. aureus*, respectively. The crude and pure extracts from *J. tanjorensis* had appreciable MICs compared with ampicillin. In addition, the anticariogenic activity of JTR3 has been reported [18].

MBC is defined as the lowest concentration of antibacterial agent that will prevent the growth of bacterium after the subculture on an antibacterium-free medium. To determine the capability of crude extracts and isolated compounds of *J. tanjorensis* in preventing the growth/kill bacterial used in this assay, we obtained their MBCs and compared them with a known reference standard (ampicillin). Table 3 indicates that JTR1 showed no MBC for all bacterial assayed, and the other extracts from *J. tanjorensis* revealed appreciable but variable MBC values compared with those of ampicillin, thus showing a synergy in its activity.

In a separate experiment for the evaluation of the synergistical attributes of α-tocopherol, a volume mixture of 1:1 naringenin and α-tocopherol was prepared and used as described earlier. The results of the experiment suggested that *E. coli*, *K. pneumoniae*, *S. typhi*, *S. pneumoniae*, and *S. aureus* had AVDZI of 10.21 ± 0.00, 11.14.00 ± 0.00, 9.9 ± 0.12, 14.00 ± 0.11, and 8.7 ± 0.13 mm, respectively. Similarly, improvement was observed in the MIC and MBC activities of the mixture toward *E. coli*, *S. pneumonia*, and *S. aureus* (Table 4). Thus, the improved antibacterial activities of *J. tanjorensis* crude extracts were due to the synergy in its antibacterial activity induced by α-tocopherol.

The research on ethnomedicinal plant-based antibacterial activity enhancers is rare, especially those specific for *J. tanjorensis*. However, studies suggested that a high α-tocopherol concentration of *Memecylon* species, including *M. malabaricum* and *M. talbotianum*, may be responsible for their superior bioactivity over other species of *Memecylon* [38]. In addition, α-tocopherols displayed potential antimicrobial benefits against methicillin-resistant *S. aureus* (MRSA) when used alone and in combinations with daptomycin. However, α-tocopherol and daptomycin amalgamation are the most effective in combating MRSA [5]. Therefore, α-tocopherol, when used in combination with a specific antibiotics, has potential antibacterial benefits. Consequently, α-tocopherol supplementation had been associated with the increased resistance to several pathogens [5]. The pharmacodynamics of synthetic and natural α-tocopherols differ [47] possibly because different phytochemicals have varying pharmacological attributes [27].

Table 2. MIC Results of *J. tanjorensis* Extracts (mg/mL)

Antibacterial Agents	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Streptococcus pneumoniae*	*Staphylococcus aureus*
MCE	2.000	2.000	16.000	4.000	2.000
HCE	16.000	1.000	16.000	8.000	8.000
CCE	32.000	2.000	32.000	16.000	4.000
MWCE	1.000	0.250	16.000	32.000	2.000
JTR1	-	-	-	-	-
JTR3	16.000	2.000	8.000	16.000	32.000
Ampicillin	0.250	0.500	1.000	0.250	0.125

- : no activity, MCE: methanol crude extract, HCE: hexane crude extract, CCE: chloroform crude extract, MWCE: methanol/water crude extract, JTR1: α-tocopherol, JTR3: naringenin.

Table 3. MBC Results of *J. tanjorensis* Extract (mg/mL)

Antibacterial Agents	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Streptococcus pneumoniae*	*Staphylococcus aureus*
MCE	4.00	4.00	32.00	8.00	4.00
HCE	32.00	2.00	32.00	16.00	32.00
CCE	64.00	4.00	64.00	16.00	8.00
MWCE	2.00	1.00	32.00	32.00	4.00
JTR1	-	-	-	-	-
JTR3	32.00	4.00	16.00	32.00	64.00
Ampicillin	0.50	1.00	2.00	1.00	0.25

- : no activity, MCE: methanol crude extract, HCE: hexane crude extract, CCE: chloroform crude extract, MWCE: methanol/water crude extract, JTR1: α-tocopherol, JTR3: naringenin.

Table 4. AVDZI, MIC, and MBC of 1:1 Mixture of JTR1 and JTR3

Antibacterial Agents	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Streptococcus pneumonia*	*Staphylococcus aureus*
AVDZI (mm)	10.21 ± 0.00	11.14 ± 0.00	9.9 ± 0.12	14.00 ± 0.11	8.7 ± 0.13
MIC (mg/mL)	8.00	2.00	8.00	8.00	16.00
MBC (mg/mL)	16.00	4.00	16.00	16.00	32.00

AVDZI: Average diameter of zone of inhibition

Therefore, the observed increase in the AVDZI of the 1:1 mixture of JTR1 and JTR3 ($\geq 8.7 \pm 0.13$ mm) against those of JTR1 ($\leq 2.23 \pm 0.12$ mm) and JTR3 ($\geq 8.1 \pm 0.13$ mm) for all assayed bacteria support our findings and thus validate the efficacy of *J. tanjorensis* leaves as a potential source of antibacterial agents for the treatment of bacterial infection in Southeast Nigeria.

Conclusion

The research on the novel functions of α-tocopherol and other tocopherol isomers has long been considered in view of understanding their bioactivities. Emerging research reveals that different α-tocopherols have peculiar pharmacodynamic profiles. The experimental results associated with the assay analysis herein showed that the antibacterial activity of *J. tanjorensis*, a flora of Southeast Nigeria, was due to the presence of naringenin in its leaf extracts, and the bio-enhanced activity of its crude extracts was attributed to the presence of α-tocopherol isolated from its crude methanol extract. The antibacterial analyses of each extract from *J. tanjorensis* have proven that these extracts are potential antibacterial agents and thus validate the ethnomedicinal efficacy of *J. tanjorensis* in the treatment of bacterial infections. Consequently, future research should gear toward the extensive assessment of the potential and efficacy of plant-based α-tocopherols as characteristic enhancers of antibacterial activity. Subsequently, clinical trials involving combinatorial therapy of plant-based α-tocopherol and known antibacterial drugs against resistant strain of bacteria should be considered.

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