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Ethnomedicinal Benefits of *Jatropha tanjorensis* and its Pharmaceuticals: An Antibacterial Study

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ABSTRACT

Ethnomedicine has been useful in the treatment of ailments caused by bacterial pathogens. Here we evaluated the antibacterial application of J. tanjorensis leaves extracts in the treatment of infections caused by E. coli, K. pneumoniae, S. typhi, S. pneumoniae and S. aureus. The results of the analysis showed that crude extracts of J. tanjorensis leaf as well as compounds isolated from them showed potential antibacterial activity and support the claim by ethnomedicine practitioners on its use in the treatment of infections caused by these pathogens.

Keywords: Antibacterial; Infections; *J. tanjorensis*; Ethnomedicine; Extracts

INTRODUCTION

Owing to increase in resistance of bacterial organisms towards synthetic drugs, there is needed to explore and exploit other alternative that poses less risk and maximum benefits. Ethnomedicine has been beneficial in the treatment of infections caused by bacterial pathogens; amongst its utmost benefits is its availability and ease of preparation; hence, serving the ever-growing population of underdeveloped nations. Resistance of bacteria organism to synthetic drugs is multifactorial though misuse and overdose of drugs is amongst the major contributing factor [1]. The plant kingdom contains library of compounds that possess medicinal benefits that are yet to be fully harnessed by man in the treatment of infectious diseases. *Jatropha* belongs to the Euphorbiaceae family which contains over 170 species, overtime *Jatropha* species have been used as ethnomedicine by native people in tropical and subtropical countries. The antibacterial activity of *Jatropha* species has been reported in specie such as *J. curcus*, *J. gossypiifolia*, *J. multifida*, *J. nana* and *J. unicostate*. Some compounds isolated from *Jatropha* species has shown antibacterial activity they include Labaditin, Curcusone C and Curcusone D, (4Z)-Jatrogrossidentadione and 15-epi-(4Z)-Jatrogrossidentadione, Multifidenol, Japodagrin 2-epi-Jatrogrossidione, 2-Hydroxyisojatrogrossidion and 2-Epihydroxyisojatrogrossidion, Fraxidin, Japodic acid and Erythrinasinate; Palmarumycins JC1, Palmarumycins JC2, Palmarumycins CP1, Jatrophenone, Japodagrone. *J. tanjorensis* has found its application in various area of ethnomedicine such as improving hematological parameters, antimalaria and antihypertensive, antioxidant, antibacterial and antidiabetic [2].

MATERIALS AND METHODS

Experimental procedure

The leaves of *J. tanjorensis* were collected from Echeaba in Ebonyi Local government area of Ebonyi state. Authentication was done by a taxonomist at the department of applied biology, Ebonyi state university as *Jatropha tanjorensis*. The leaves were air dried and pulverized with the aid of both mechanical grinder and mortar and kept for further use in an air tight container. All chemicals used were sigma aldrich quality grade, melting point was determined using a duran thiele apparatus while the structure of isolated compound were determined using Bruker 500 MHz spectroscopy. All bacteria used were local clinical isolates while the ampicillin used was Cikacillin® (Ampicillin Trihydrate BP 250 mg). Statistical analysis were done using SPSS version 20 [3].

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). 10 kg of the pulverized leaves of each plant part was weighed and soaked in the

appropriate solvents in order of increasing polarity for 72 hours. The mixture was filtered, and the filtrate were concentrated using a rotary evaporator (Stuart RE 300/MS, UK) to one-tenth of its volume at 40°C. Each dried extract was weighed in an analytical balance (OHAUS PX225D, USA) and stored at -4°C [4].

Column and flash chromatographic separation: In each, 15 g of the crude extract was subjected to column chromatography and eluted with Hex-EtOAc (80:20, 70:30, 60:40, 50:50.), EtOAc (100%) and MeOH (100%) gradients. The leaf extracts (15 g) was dissolved in the eluting solvent and packed on top of the silica gel slurry with a pipette, and then the eluting solvent was added. Collection of the eluent was done with 50 mL and 100 mL conical flasks. Further elution was done with increasing concentration gradients. For the MeOH leaf crude extract, elution was carried out using DCM-EtOAc (80:20, 70:30), EtOAc (100%), EtOAc-MeOH (50:50) and MeOH (100%) gradients. For n-Hex leaf crude extract, elution was done with Hex-DCM gradients (60:40, 50:50), EtOAc (100%), EtOAc-MeOH (50:50), and MeOH-H_2O (80:20). For $CHCl_3$ leaf crude extract, elution was done with Hex-DCM gradients (60:40, 50:50), EtOAc (100%), EtOAc-MeOH (50:50), and finally with 100% MeOH. Elution of MeOH-H_2O leaf extract was carried out with DCM-EtOAc (80:20), EtOAc (100%), MeOH (100%) [5]. The fractions from MeOH extracts were labelled JTR1 and JTR3. Fractions collected were monitored for purity by spotting on Thin Layer Chromatographic (TLC) plates. Further purification was carried out using flash chromatographic technique using a solvent system of PET-$CHCl_3$. Concentration, drying and washing of the fractions severally with methanol afforded pure extracts labelled JTR1 and JTR3. The pure fractions were further recrystallized three times, weighed and stored for use at 4°C [6].

Evaluation of antimicrobial activity

Determination of diameter of zone of inhibition: Clinical isolates of the assayed bacteria organisms were obtained from Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA). The Isolates were tested for viability by resuscitating them in buffered peptone water after which they were subcultured into nutrient agar medium and incubated at 37°C for 24 hrs. The organisms were then stored at 4°C until when needed. Agar well diffusion techniques as described, was adopted for the study. 18 mL of Mueller Hinton Agar plates (MHA oxoid) England, were inoculated with 0.1 mL of an overnight broth culture of each bacteria isolate (Equivalent to 3×10^7 cfu/mL) MF (Mcfarland standard) in sterile petri-dish [7]. Holes were bored on the plates by using standard sterile cork borer of 6 mm diameters and equal volumes of the proposed antimicrobial agent (1000 µL) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The control experiments were setup with 1000 µL of 70% methanol in separate welled. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract. The plates were incubated at 37°C for 24 h until marked decline in the potency of both crude and pure extracts to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded [8].

Determination of the minimum inhibitory concentration: The method described was used in the determination of the Minimum Inhibitory Concentration (MIC) was carried out on extracts and isolates that showed sensitivity against the growth of the test organisms. The medium used was Mueller Hinton Agar solution which was prepared according to the manufacturer's standard of 38 g/1000 mL double strength was prepared by dissolving 38 g in 500 mL of distilled water, homogenized and 5 mL was dispensed into 40 sets of McCartney bottles and sterilized in an autoclave at 121°C for 15 min [9]. The agar was allowed to cool to 45°C and each graded solution was then poured into petri-dishes and allow to solidity for one hour. Extracts concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/mL were prepared by serial dilution. Each plate was divided into 4 (four) equal section and labeled accordingly. The 5 mm diameter paper discs were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 mL of each bacterial suspension was taken and transferred aseptically into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37°C after which they were observed for growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the Minimum Inhibitory Concentration (MIC) [10].

Determination of the minimum bactericidal concentration: The determination of MBC was carried out by preparing 40 sets of plates of Mueller Hinton agar and sterilized. 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 sub-cultured into appropriately labeled quadrants of the sterilized Mueller Hinton agar plates. The organisms were uniformly streaked on labeled quadrants using wire loop. The organisms were incubated at 37°C for 24 hours, after which growth were observed and recorded. The MBC was the quadrant with lowest concentration of the extract without growth [11].

RESULTS AND DISCUSSION

Results of spectral elucidation

JTR1: *α*-tocopherol: Yellowish-brown oil; R_f 0.66; yield 0.28%; melting point 2°C-3°C; 1 H-NR (500 MHz, $CDCl_3$): δ_H 7.18 ppm (6-OH), 2.38 ppm (H-4), 1.55 ppm (H-3'), 2.16 ppm (H-1'), 2.11 ppm (H-2'), 1.55 ppm (H-3), 1.49 ppm (H-11) 1.24-1.39 (H-12, H-14, H-18), 1.20-1.24 ppm (H-13, H-15, H-16, H-20), 1.10 ppm (H-4'), 1.04-1.08 (H-17, H-19, H-21, H-22), 0.73-0.76 (H-5', H-6', H-7', H-23). ^{13}C-NMR (500 MHz, $CDCl_3$): δ_C 145.52 ppm (C-6), 145.54 ppm (C-9), 122.61 ppm (C-8), 117. 35 ppm (C-10), 121.00 ppm (C-7), 118.46 ppm (C-5), 74.52 ppm (C-2), 39.87 ppm (C-11), 37.46 ppm (C-13), 37.39 ppm (C-15), 37.29 ppm (C-17), 32.79 ppm (C-19), 39.37 ppm (C-21), 32.70 ppm (C-14), 31.54 ppm (C-18), 19.69 ppm (C-4), 27.98 ppm (C-22), 24.82 ppm (C-20), 27.98 ppm (C-22), 22.64 ppm (C-23), 20.77 ppm (C-12), 19.65 ppm (C-5'), 19.60 ppm (C-6'), 31.48 ppm (C-3), 11.29 ppm (C-1'), 12.22 ppm (C-2'), 11.79 ppm (C-3'), 23.80 ppm (C-4'), 22.74 ppm (C-7'). NMR spectra analysis confirmed the structure as α-tocopherol. Calc. molecular mass is 430.71 g/mol (Figure 1) [12].

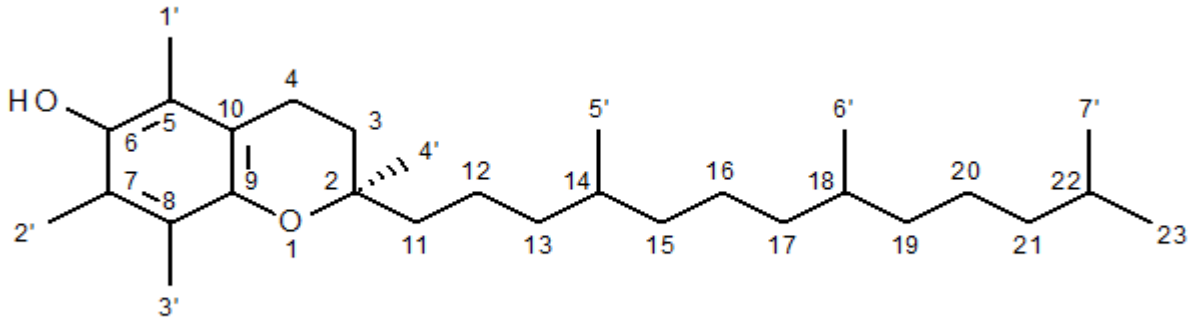

Figure 1: α- tocopherol ($C_{29}H_{50}O_2$).

JTR 3: Naringenin: White solid; R_f 0.81; yield 0.10 %; melting point 253°C -255°C; ^{13}C (500 MHZ, DMSO-d6): C-2 (80.51 ppm), C-3 (42.06 ppm), C-4 (197.83 ppm), C-5 (165.50 ppm), C-6 (97.06 ppm), C-7 (165.50 ppm), C-8 (96.18 ppm), C-9 (168.36 ppm), C-10 (103.36 ppm), C-1' (131.09 ppm), C-2' (129.10 ppm), C-3' (116.33 PPM), C-4' (159.06 PPM), C-5' (116.32), C-6' (129.32). 1H (500 MHZ, DMSO-d_6): H-2 (5.30 ppm), H-3 (α-3.10, β-2.69), H-6 (5.90 ppm), H-8 (5.89 ppm), H-2' (7.30 ppm), H-3' (6.80 ppm), H-5' (6.83 ppm), H-6' (7.33 ppm). NMR spectra analysis for JTR3 matched those of Naringenin and was thus assigned. Calc. molecular mass is 272.25 g/mol (Figure 2) [13].

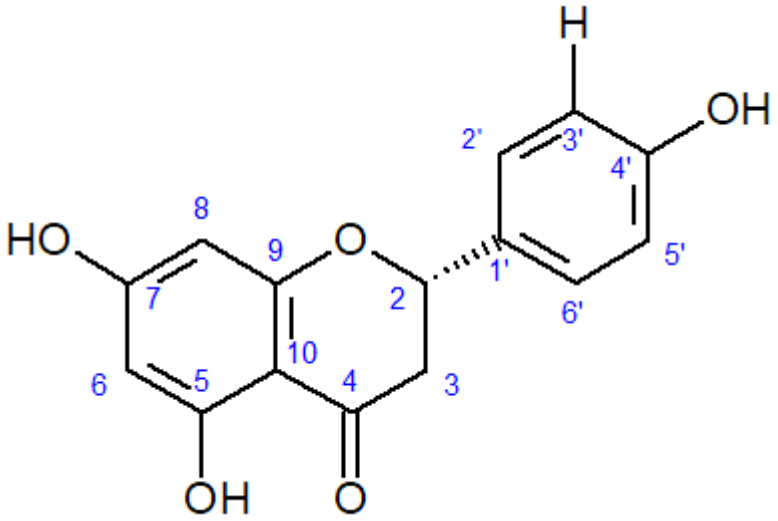

Figure 2: Naringenin ($C_{15}H_{12}O_5$).

Results of antibacterial analysis

Table 1 depicts the average diameter of zone of inhibition for the various extracts of *J. tanjorensis*. Though ampicillin showed better activity for all bacteria assayed, the average diameter of zone of inhibition for all extracts except JTR3 were quite comparable. They have been previous report on the synergistic antimicrobial activity of JTR1. The antibacterial activity of JTR3 as well as *Jatropha tanjorensis* has been profiled. While, established the antimicrobial properties of *Jatropha tanjorensis* attributed the broad spectrum antimicrobial activities of *Jatropha tanjorensis* to the presence of phytochemicals isolated from its methanol extract reported the antimicrobial activities of *Jatropha curcas* a specie of the *Jatropha* genus indicating the wide antimicrobial application of *Jatropha* species in ethnomedicine showed the improved antimicrobial activity and potentiation of aminoglycosides by alpha-tocopherol against Multiresistant bacteria, thus the improved antimicrobial activities of *Jatropha tanjorensis* crude extracts is due to the synergy in its antimicrobial activity.

Table 1: Results of average diameter of zone of inhibition of *J. tanjorensis* extracts.

Antibacterial agents	***E. coli***	***K. pneumoniae***	***S. typhi***	***S. pneumonia***	***S. 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}
Note: * "a, b, c, d, e, f, g" depicts the various level of significance between the antimicrobial agents obtained from tukey posthoc analysis. Distinct letters in the same column indicates significance difference ($p<0.05$) while similar letters in the same column shows non-significance difference ($p>0.05$). Letters with * indicates that such groups are significantly different from each other but not significantly different from other antimicrobial agents.					

From Table 2, MWCE gave MIC of 1.000 mg/mL and 0.250 mg/mL for *E. coli* and *K. pneumoniae* respectively, CCE gave MIC of 32.000 mg/mL for both *E. coli* and *S. typhi* [14]. JTR1 showed no MIC at coverage concentration for all bacteria analyzed while MCE, HCE and MWCE were 16.000 mg/mL for *S. typhi* [15]. MCE had MIC for *E. coli, K. pneumoniae, S. typhi, S. pneumoniae* and *S. aureus* at 2.000 mg/mL, 2.000 mg/mL, 16.000 mg/mL, 4.000 mg/mL and 0.250 mg/0 mL respectively. Excluding JTR1 both the crude and pure extracts had appreciable MIC when compared to ampicillin [16].

Table 2: MIC results of *J. tanjorensis* extracts.

Antibacterial agents	***E. coli***	***K. pneumoniae***	***S. typhi***	***S. pneumoniae***	***S. aureus***
MCE	2	2	16	4	2
HCE	16	1	16	8	8
CCE	32	2	32	16	4
MWCE	1	0.25	16	32	2
JTR1	-	-	-	-	-
JTR3	16	2	8	16	32
Ampicillin	0.25	0.5	1	0.25	0.125

Table 3 indicated that JTR1 showed no MBC for all bacterial assayed though other extracts from *J. tanjorensis* showed appreciable but variable MBC value when compared to those of ampicillin thus showing a synergy in its activity [17].

Table 3: MBC results of *J. tanjorensis* extract.

Antibacterial agents	***E. coli***	***K. pneumoniae***	***S. typhi***	***S. pneumoniae***	***S. aureus***
MCE	4	4	32	8	4
HCE	32	2	32	16	32
CCE	64	4	64	16	8
MWCE	2	1	32	32	4
JTR1	-	-	-	-	-
JTR3	32	4	16	32	64
Ampicillin	0.5	1	2	1	0.25

CONCLUSION

The results of antibacterial analysis of extracts from *J. tanjorensis* has proven that these extracts are potential antibacterial agents and thus validates its ethnomedicinal use in the treatment of infections caused by bacterial used in this assay. While, established the antimicrobial properties of *Jatropha tanjorensis* attributed the broad spectrum antimicrobial activities of *Jatropha tanjorensis* to the presence of phytochemicals isolated from its methanol extract reported the antimicrobial activities of *Jatropha curcas* a specie of the *Jatropha* genus indicating the wide antimicrobial application of Jatropha species in ethnomedicine showed the improved antimicrobial activity and potentiation of aminoglycosides by alpha-tocopherol against Multiresistant bacteria, thus the improved antimicrobial activities of *Jatropha tanjorensis* crude extracts is due to the synergy in its antimicrobial activity. Similarly, the increased antibacterial activity of its crude extracts over the pure extracts indicates synergy in its antibacterial effect.

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