

**STUDIES ON *Dacryodes edulis* III: ISOLATION AND CHARACTERIZATION OF GALLIC ACID FROM METHANOLIC EXTRACT OF RAW (UNTREATED) SEEDS OF *Dacryodes edulis* AND ITS ANTIMICROBIAL PROPERTIES**

**\*T. A. Tor-Anyiin, J. O. Igoli, and J. N. Anyam**

Phytochemistry Research Group, Department of Chemistry, University of Agriculture, Makurdi, Nigeria.

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\*Corresponding Author: [toranyiint@yahoo.com](mailto:toranyiint@yahoo.com)

**Abstract**

*In continuation of our studies on seeds of D. edulis, the methanol extract of raw/untreated seeds of Dacryodes edulis was subjected to vacuum liquid chromatography (VLC) on silica gel. Needle shaped crystals were observed. Fractions were monitored using TLC and similar fractions were combined and further purified using Sephadex LH-20 column (gel filtration chromatographic technique). This led to the isolation of gallic acid, as needle shaped crystals which was characterized by FT-IR, <sup>1</sup>H and <sup>13</sup>C-NMR spectra data as well as comparison with reported data. Gallic acid showed moderate antibacterial and antifungal activities. This is the first report on isolation of gallic acid from the seeds of Dacryodes edulis.*

**Key Words:** *Dacryodes edulis*, Raw/Untreated seeds, Methanol extract, Gallic acid, Antimicrobial activity.

**Introduction**

Many natural products have been isolated and identified as potential medicinal agents. Isolation of compounds with novel structural features with promise of being used as drugs of better therapeutic properties continues today [1].

With the good promise of our earlier reports [2 and 3] in view, we have continued our studies on the seeds of *D. edulis*. Thus, we report herein isolation and characterization of gallic acid from methanol extract of untreated/raw seeds of *D. edulis* as well as its antimicrobial activity.

**Materials and Methods**

**Plant Material**

Fruits of the plant were collected from Iyonov in Kwande local government area Benue state, Nigeria in July 2013. It was identified and authenticated at the Department of Wildlife and Range Management, University of Agriculture, Makurdi. Fruits were dehulled to obtain seeds which were pulverized with a mortar and pestle after which they were sequentially extracted using hexane, chloroform, ethyl acetate and methanol by way of maceration.

**Extraction and Isolation**

Extraction was as earlier reported [2]. Pulverized plant material (5 kg) was defatted with hexane (10 Litres), and then sequentially extracted with chloroform (8 Litres), ethyl acetate (8 Litres) and methanol (10 Litres) for 72 hrs each by way of cold maceration. All extracts were concentrated using a rotary evaporator at 40 °C (50 °C for Methanol). The methanol extract was subjected to Vacuum Liquid Chromatography (VLC) using

gradient elution (from 100 % hexane through to 100 % ethyl-acetate and from 100 % chloroform through to 100 % methanol). Fractions were subjected to thin-layer chromatography (TLC) using chloroform-acetone-formic acid (7.5:1.65:0.85) and toluene-ethyl acetate-formic acid (5:4:1). Similar fractions (with same R<sub>f</sub> value) were combined and further purified by gel filtration (Sephadex LH-20). Methanol was used as solvent of elution and fractions were monitored with TLC. Chloroform-acetone-formic acid (7.5:1.65:0.85) and toluene-ethyl acetate-formic acid (5:4:1) were the solvent systems used for TLC. Fraction 14 which crystallized as fine needle shaped was further subjected to antimicrobial assay and spectroscopic analysis (FT-IR, <sup>1</sup>H and <sup>13</sup>C-NMR). FT-IR (Thermo Fisher Scientific FT-IR Machine (Nicolet IS10); <sup>1</sup>H and <sup>13</sup>C-NMR (JEOL JNM-ECS-400MHz (for <sup>1</sup>H) and 101MHz (<sup>13</sup>C) were used.

**Antimicrobial Study**

Sensitivity test was carried out using agar diffusion method. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) were carried out using broth dilution method. This were done similarly as earlier reported [2] using the following microorganisms: *Staphylococcus aureus*; *Streptococcus pyogenes*; *Escherichia coli*; *Salmonella typhi*; *Shigella dysenteriae*; *Pseudomonas aeruginosa*; *klebsiella pneumoniae*, *Candida albicans*; *Candida tropicalis*; *Candida thrusei*; *Candida stellatoidea*; *Microsporum gypseum*; *Microsporum sp.*, and *Trichophyton rubrum* obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria.

## Results and Discussion

Fraction 14 from gel filtration chromatography gave white needle shaped crystals weighing 200 mg on drying at ambient temperature. The melting point was determined to be 240 °C with decomposition. The crystals showed significant antimicrobial activity against tested microbes. They were sensitive against 8 microbes (*S. aureus*, *C. tropicalis*, *C. thrusei*, *C. stellatoidea*, *S. pyogenes*, *S. dysenteriae*, *K. pneumoniae* and *C. albicans*) as compared to the antibiotic ciprofloxacin which was sensitive against 7 microbes (*S. aureus*, *S. pyogenes*, *E. coli*, *S. typhi*, *S. dysenteriae*, *P. aeruginosa* and *K. pneumoniae*); antifungal fulcin was sensitive against *M. gypseum*, *Microsporum sp.* and *T.*

*rubrum* while antifungal fluconazole was sensitive against *C. albicans*, *C. tropicalis*, *C. thrusei* and *S. stellatoidea*.

From diameters zone of inhibition, fraction 14 exhibited highest inhibitory effect for *K. Pneumoniae* (29 mm) with MIC values of 2.5 µg/mL for *S. aureus*, *C. tropicalis*, *C. thrusei* and *C. stellatoidea*; and 1.25 µg/mL for *S. pyogenes*, *S. dysenteriae*, *K. pneumoniae* and *C. albicans*. It also exhibited MBC/MFC values of 10 µg/mL for *S. aureus*, *C. tropicalis*, *C. thrusei* and *C. stellatoidea*; 5µg/mL for *S. pyogenes*, *S. dysenteriae* and *C. albicans*; and 2.5 µg/mL for *K. pneumoniae*. (Table 1).

**Table 1. Antimicrobial activity of fraction 14**

|                        | Sensitivity |               |        |             | Zone of Inhibition (mm) |              |        |             | Minimum Inhibitory Concentration(MI C) |           |            |             | Minimum Bactericidal/Fungicidal Concentration(MBC/MFC) |         |           |            |            |
|------------------------|-------------|---------------|--------|-------------|-------------------------|--------------|--------|-------------|----------------------------------------|-----------|------------|-------------|--------------------------------------------------------|---------|-----------|------------|------------|
| Pathogen               | Fraction 14 | Ciprofloxacin | Fulcin | Fluconazole | Fraction 14             | Ciprofloxaci | Fulcin | Fluconazole | 5 µg/mL                                | 2.5 µg/mL | 1.25 µg/mL | 0.625 µg/mL | 10 µg/mL                                               | 5 µg/mL | 2.5 µg/mL | 1.25 µg/mL | 0.62 µg/mL |
| <i>S. aureus</i>       | S           | S             | R      | R           | 23                      | 35           | 0      | 0           | -                                      | α         | +          | ++          | β                                                      | +       | +         | ++         | +++        |
| <i>S. pyogenes</i>     | S           | S             | R      | R           | 27                      | 37           | 0      | 0           | -                                      | -         | α          | +           | -                                                      | β       | +         | ++         | +++        |
| <i>E. coli</i>         | R           | S             | R      | R           | 0                       | 39           | 0      | 0           |                                        |           |            |             |                                                        |         |           |            |            |
| <i>S. typhii</i>       | R           | S             | R      | R           | 0                       | 41           | 0      | 0           |                                        |           |            |             |                                                        |         |           |            |            |
| <i>S. dysenteriae</i>  | S           | S             | R      | R           | 27                      | 44           | 0      | 0           | -                                      | -         | α          | +           | -                                                      | β       | +         | ++         | +++        |
| <i>P. aeruginosa</i>   | R           | S             | R      | R           | 0                       | 32           | 0      | 0           |                                        |           |            |             |                                                        |         |           |            |            |
| <i>K. pneumoniae</i>   | S           | S             | R      | R           | 29                      | 40           | 0      | 0           | -                                      | -         | α          | +           | -                                                      | -       | β         | +          | ++         |
| <i>C. albicans</i>     | S           | R             | R      | S           | 28                      | 0            | 0      | 32          | -                                      | -         | α          | +           | -                                                      | γ       | +         | ++         | +++        |
| <i>C. tropicalis</i>   | S           | R             | R      | S           | 22                      | 0            | 0      | 37          | -                                      | α         | +          | ++          | γ                                                      | +       | +         | ++         | +++        |
| <i>C. thrusei</i>      | S           | R             | R      | S           | 23                      | 0            | 0      | 35          | -                                      | α         | +          | ++          | γ                                                      | +       | +         | ++         | +++        |
| <i>C. Stellatoidea</i> | S           | R             | R      | S           | 25                      | 0            | 0      | 32          | -                                      | α         | +          | ++          | γ                                                      | +       | +         | ++         | +++        |
| <i>M. gypseum</i>      | R           | R             | S      | R           | 0                       | 0            | 32     | 0           |                                        |           |            |             |                                                        |         |           |            |            |
| <i>Microsporums</i>    | R           | R             | S      | R           | 0                       | 0            | 31     | 0           |                                        |           |            |             |                                                        |         |           |            |            |
| <i>pp</i>              |             |               |        |             |                         |              |        |             |                                        |           |            |             |                                                        |         |           |            |            |
| <i>T. rubrum</i>       | R           | R             | S      | R           | 0                       | 0            | 34     | 0           |                                        |           |            |             |                                                        |         |           |            |            |

Key: S = Sensitive, R = Resistant, - = (No turbidity) No colony growth, α = MIC, β = MBC, γ = MFC + = (Turbid) Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth

IR spectra of fraction 14 showed frequencies due to OH stretching vibrations of both phenolic (3495.03 cm<sup>-1</sup>, 3404.38 cm<sup>-1</sup>, 3389.24 cm<sup>-1</sup>) as well as hydroxyl group of carboxylic acid (3279.04 cm<sup>-1</sup>). The phenolic hydroxyl stretching vibrations are usually observed at higher wave numbers than the -OH stretching vibrations

of carboxylic group (COOH) [4]. The C=O stretching band of moderate intensity was observed as a sharp band at vibrational wave number 1668.03 cm<sup>-1</sup>. Inter or intramolecular hydrogen bonds may account for why band for the C=O group was observed at 1668.03 cm<sup>-1</sup> as it is one of the factors that determine the position of C=O stretching band. Also, hydrogen bonding and

resonance weaken the C=O bond resulting in absorption at lower wavenumber than the monomer (which absorbs at 1710 cm<sup>-1</sup>) [4].

This result compares well with report by Hirun *et al.* [5] (which showed four component bands for the hydroxyl groups at 3647, 3607, 3540 and 3353 cm<sup>-1</sup>; and 1668 cm<sup>-1</sup> for C=O stretching band of the carboxylic acid) in that they fall within absorption range (3700-3584 cm<sup>-1</sup>/ 3550-3200 cm<sup>-1</sup> for OH of hydroxyl group of phenols and alcohols) as stated in literature [4].

The <sup>1</sup>H-NMR spectra recorded in DMSO-d<sub>6</sub>, showed one signal at chemical shift δ 6.86 due to a set of chemically equivalent protons at H-2 and H-6). The signal at δ 6.86 was due to the aromatic protons; a result of electron releasing substituents (OH) at positions 3 and 5 is consistent with reported data on gallic acid [6 and 7].

<sup>13</sup>C-NMR spectra recorded in (101MHz, DMSO-D<sub>6</sub>), gave five signals with two sets of equivalent carbons. The chemical shifts were; δ167.81 (C-7), 145.59 (C-3 & 5), 138.35 (C-4), 120.61 (C-1) and 108.78 (C-2 & 6). C-7 is deshielded as it is a carbonyl group. This agrees with reports from literature [6 and 7]. (Table 2).

**Table 2.** <sup>13</sup>C-NMR spectral data of fraction 14 in DMSO-d<sub>6</sub> (δ in ppm) in comparison with literature.

| Position | Experimental | Literature |       |
|----------|--------------|------------|-------|
|          |              | A          | B     |
| 1        | 120.61       | 120.8      | 121.0 |
| 2 & 6    | 108.78       | 109.1      | 109.0 |
| 3 & 5    | 145.59       | 145.6      | 145.9 |
| 4        | 138.35       | 138.2      | 138.3 |
| 7        | 167.81       | 167.8      | 168.0 |

KEY: A- [7] B- [6]

Comparison of FT-IR, <sup>1</sup>H and <sup>13</sup>C-NMR spectra data with reported data, reveals fraction 14 as gallic acid.

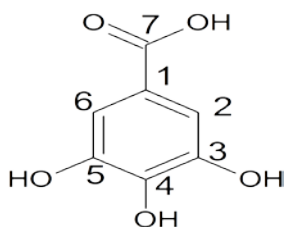


**Figure 1.** Gallic acid

Gallic acid is also known as 3, 4, 5- trihydroxybenzoic acid. Its derivatives have been reported in many phytomedicines with a number of biological and pharmacological activities which include; scavenging on free radicals [8], apoptosis of cancer cells [9, 10 and 11]. Report has shown that gallic acid is an effective inhibitor of glucoamylase and therefore may be a hypoglycaemic agent [1]. Gallic acid has been reported to possess antibacterial and antifungal properties [12 and 13]. This is in agreement with our findings.

## Conclusion

Gallic acid was isolated from the untreated/raw seeds of *Dacryodes edulis*. It demonstrated antibacterial and antifungal properties. This study may be considered as the first report on isolation of gallic acid from the seeds of *D. edulis* and any morphological part of this plant.

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