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Research Article

Isolation and Characterization of Geranylated Chalcone from Ethylacetate Fraction of *Terminalia brownii* Fresen (Combretaceae)

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Abstract

Terminalia brownii is a deciduous tree characterized by spirally arranged leaves concentrated at the ends of its branches. The plant is widely distributed in Northern Nigeria and other part of Africa, including Congo, Kenya, Sudan, Ethiopia, and Tanzania. Traditionally, it is used for treating various conditions such as gastric ulcers, epilepsy, colitis, jaundice, fungal infections, diarrhea, malaria, hepatitis, and allergic reactions. This study aimed to analyzed the crude methanol leave extract and ethyl acetate fraction of *Terminalia brownii* to isolate secondary metabolites. Plant material was collected, air-dried, pulverized, and extracted using cold maceration with 70% methanol. The resulting crude methanol extract was fractionated with n-hexane, chloroform, ethyl acetate, and n-butanol to yield their respective fractions. The ethyl acetate underwent extensive column chromatography using silica gel and Sephadex LH-20. Structural characterization of the isolated compound was conducted through physical and chemical analysis, UV and IR spectroscopy, advanced 1D and 2D NMR techniques, supported by literature references. This process resulted in the isolation of a yellow amorphous compound, identified as 2',6',4-trihydroxy-3'-methoxy-4-O-prenyloxy chalcone (geranylated chalcone). The compound is isolated for the first time from *Terminalia brownii* leave, this discovery convey *Terminalia brownii* as a rich source of secondary metabolites and contributes to the taxonomy of the plant.

Keywords

Metabolites, *Terminalia brownii*, Isolation, IR, UV

1. Introduction

Terminalia brownii Friesen, commonly known as the red pod Terminalia, belongs to the family Combretaceae, a significant group of angiosperms. Its classification was first established by Robert Brown in 1810, placing it under the order Myrtales [2]. The species thrives predominantly in tropical and subtropical regions. In Africa, it is widely distributed across Northern Nigeria, as well as Kenya, Tanzania, Ethiopia, Sudan, and the Democratic Republic of Congo [6].

Various parts of the plant, including the stem, roots, wood, and leaves, have been extensively used in traditional medicine

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to manage conditions such as rheumatic and back pain, hepatitis, toothache, tonsillitis, typhoid, snake bites, bacterial and fungal infections, endometriosis, gonorrhea, jaundice, malaria, stomach aches, ulcers, and sexually transmitted diseases [5]. Additionally, it is used cosmetically as a local perfume for women [3]. The leaves are used by traditional healers in Tanzania to treat diarrhea and stomach ache, gastric ulcers, colic, and heartburn [7]

Previous studies have reported the isolation of several secondary metabolites from the stem bark using hexane, including sitosterol, stigmasterol, Monaogynol A, betulinic acid, arjungenin, arjunic acid, and various methylellagic acid derivatives [3]. Extracts from different parts of *T. brownii* exhibit diverse biological activities, including antimicrobial, antiallergic, anti-inflammatory, antinociceptive, radical scavenging, and anticancer properties, with the stem wood showing notable anticancer potential [3].

In 2020, Ahmed and Almagboul isolated active constituents such as gallic acid, dihydroxy flavone, and kaempferol 7-methoxy-3-sulfate from the methanolic leaf extract of *T. brownii*. Furthermore, methanol extracts derived from dried leaves demonstrated significant antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* [1]. These findings highlight the therapeutic potential and diverse pharmacological activities of *Terminalia* brownii [4]. Kareru *et al* in 2008, reported the highly sensitivity of leaves and back extract of *Terminalia brownii* to treat *Escherichia coli* and *staphylococcus aureus* [4].

2. Materials and Methods

2.1. Plant Material

The leaf part of *Terminalia brownii* were collected from Maraba Yakawada Giwa local government Zaria Kaduna State, Nigeria in March, 2022. The plant sample was authenticated at the Herbarium Section of Botany department, Ahmadu Bello University, Zaria, through comparison with herbarium reference (ABU0406). The leave part of the plant was rinsed, air-dried to constant weight and size reduced using pestle and mortar and subsequently referred to as powdered plant material.

2.2. Extraction and Fractionation

About 700 g of the powdered plant material was extracted with 70% methanol using cold maceration method for 72 hours and tilted occasionally. The extract was separated from the shaft with filter paper and the solvent was recovered using rotary evaporator to give 92g concentrated crude methanol extract with percentage yield of 20%. 85g of the crude extract was partitioned using n-hexane, chloroform, ethyl acetate and n-Butanol respectively to obtain 10.18 g of n-hexane fraction chloroform fraction (3.6 g), ethyl acetate fraction (8 g), and n-Butanol fraction (36 g). The ethyl acetate fraction was subjected to column chromatographic analysis for isolation.

2.3. Isolation of Compound T4 from Ethyl Acetate Fraction Terminalia Brownii

Approximately 6 g of the ethyl acetate fraction (EAF) was subjected to column chromatography using a glass column measuring 75 cm in height and 3.5 cm in diameter. Silica gel (60-120 mesh) was used as the stationary phase, and the fraction was eluted with a gradient of hexane and ethyl acetate. The gradient started with 100% hexane, followed by increasing proportions of ethyl acetate (Hex: EA 95:5, Hex: EA 90:10, etc.) until the column was finally flushed with 100% methanol. The progress of the column was monitored using pre-coated thin-layer chromatography (TLC) plates in a one-way ascending technique with various solvent systems. Spots on the TLC plates were visualized under UV light (254-366 nm), and the chromatograms were developed by spraying with 10% sulfuric acid followed by heating at 110 ℃ in an oven. A total of 78 eluates, each 2 mL in volume, were collected. Fractions with similar TLC profiles were combined to form 11 bulk fractions, labeled T1 to T11. Bulk fraction T4 was further purified using Sephadex LH-20, yielding a single homogeneous spot on TLC, confirming its purity.

3. Result and Discussion

The crude methanol leave extract of Terminalia brownii was fractionalized into n-hexane, Chloroform, Ethylacetate and n-butanol fractions and they were spotted on a TLC plate. Ethylacetate fraction showed a good TLC profile and was subjected to Column chromatographic separation. The fraction was eluted from the column with mobile phase in a gradient manner starting with 100% n-Hexane, followed by Hex: EA 95:5 the polarity was gradually increased till the column was washed with methanol. 118 eluents of 50ml each was collected, pooled and coded Tb1- Tb10 base on their TLC profile similarities. The pooled collections Tb10 was purified with sephadex LH-20 and this led to the isolation of a yellow amorphous substance coded T4. T4 gave a single homogeneous spot on TLC with three different solvent system H: EA 3ss:7, DCM: EA 4:6 and DCM: EA 1:1 and the following R_f value 0.63, 0.78 and 0.72 (Figure 1). T4 was completely soluble in methanol and Dichloromethane, melted at 122-125 ℃ and tested positive to ferric chloride test as suggested by [8]. The nuclear magnetic resonance spectroscopy of T4, revealed the presence of one compound suggested in (Table 1).

Figure 1. *TLC profile of T4.*

3.1. Elucidation of Compound

The physicochemical properties of the compound such as solubility, chemical test, and melting point was determined. The structure of the pure compound isolated was established with UV, IR, 1D NMR (Shimadzu 8400S FTIR spectrometer), and Nuclear Magnetic Resonance Spectroscopic (Bruker AVANCE III Instruments Incorporation, Billerica, MA, USA 400 MHz) (1D and 2D) and literatures. The proposed structure was established using chemdraw.

Table 1. *^{1}H and ^{13}C-NMR ($CDCl_3$, 400MHz) spectral data for compound T4.*

Position	$\delta_{H\ (ppm)}$	$\delta_{C\ (ppm)}$
1	---	129.0
2	7.13 (dd, J=2.7, 8.40Hz)	130.60
3	7.54 (dd, J=2.7, 8.10Hz)	114.31
4	OH	165.40
5	7.54 (m)	114.31
6	7.13 (dd, J=2.7, 8.40Hz)	130.60
1’	---	112.50
2’	OH	168.10
3’	3.66 OCH_3	51.60
4’	O- prenyl	158.51
5’	6.99	105.10
6’	OH	132.20
1”	C=O	196.50
2”	7.73	144.0
3”	7.37	116.5
1”’	3.33	65.20
2”’	2.80	25.80
3”’	4.96	118.40
4”’	5.77	139.20
5”’	1.60	18.5
6”’	1.29	28.12
7”’	0.87	12.78

3.2. Discussion

The UV spectrum of compound T4 (Figure 2) recorded in ethanol showed absorption maxima at 205.13 nm and absorbance of 3.939 indicating the presence chromophore. The FT-IR spectral data of T4 (Figure 3) showed frequency vibration at 3477.93 which is due to OH stretching, a symmetrical and asymmetrical aliphatic stretching vibration at 2922.85 and 2851.488. Frequency signal at 1673.89 is due to carbonyl stretching and also showed signal for aromatic C=C at 1509.61.

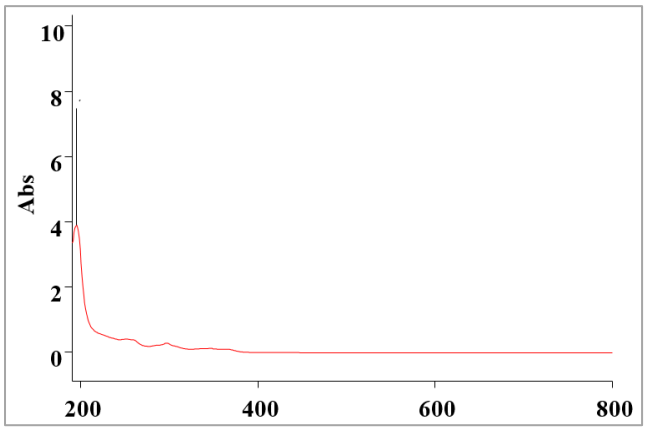

Figure 2. *UV spectrum of T4.*

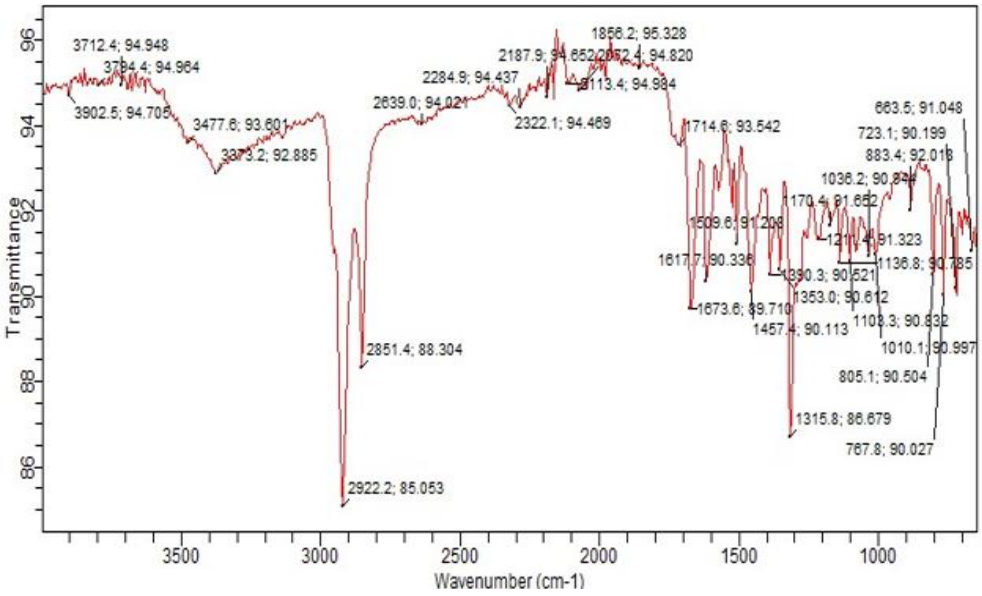

Figure 3. *FT-IR of compound T4.*

The ^{1}HNMR spectrum of compound T4 showed resonance signals at 7.72 (1H, m, H-2’’), 7.34 (1H, m, H-3’’), 7.54 (2H, dd, J=2.7,8.10Hz, H-3,5), 7.13 (2H, dd, J= 2.7, 8.40Hz, H-2), 6.99(1H, s, H-5), 5.77 (1H, m, H-4’’’’), 4.96 (1H, m, H-3’’’), 3.66 (3H, s, OCH_3-3’), 3.33 (2H, m, OCH_2-1’’’), 2.80 (2H, m, CH_2-2’’’) 1.60 (3H, s, prenyl-CH_3), 1.26 (1H, m, prenyl-CH), 0.86 (3H, s, prenyl-CH_3). C-13NMR spectrum revealed 23 signals, (C-1) 129.0, (C-2 and C-6) 130.60, (C-3 and C-5) 114.31, (C-4) 165.40,,, (C-1’) 112.50, (C-2’) 168.10, (C-3’) 51.60, (C-4’) 158.51, (C-5’) 105., (C-6’) 132.20, (C-1’’) 196.50, (C-2’’) 144.0, (C-3’’) 116.50, (C-1’’’) 65.20, (C-2’’’) 25.80, (C-3’’’) 118.40, (C-4’’’) 139.20, (C-5’’’) 18.50, (C-6’’’) 28.12, (C-7’’’) 12.78. The COSY NMR spectrum of compound T4 revealed the correlation of protons in the same chemical environment. The following are H-H correlation of compound T4 (2”, 5), (5, 2), (2, 3), (5, 6), (3”, 6), (2”’, 1”’), (3”’, 4”’) (6’’’, 5’’’), 2’,6’,4-trihydroxy-3’-methoxy-4-O-prenyloxy chalcone.

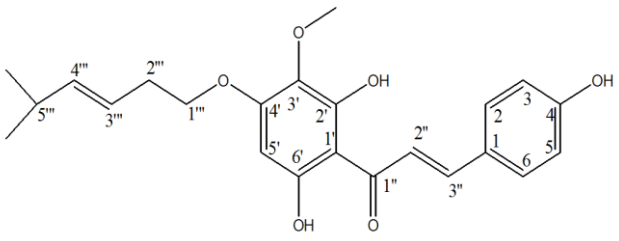

Figure 4. *Proposed structure of compound T4 (Geranylated chalcone).*

Chalcones, or 1,3-aryl-2-propene-1-ones, are part of the flavonoid family and represent open-chain flavonoids where two aromatic rings are connected by an α,β-unsaturated carbonyl system. There is growing interest in the health benefits of phytochemicals, particularly prenylated flavonoids, due to their notable biological activities. Structure-activity relationship (SAR) studies have highlighted that the bioactivity of prenylated flavonoids is largely influenced by the presence of isoprenoid chains, which vary in length and type. These chains enhance the compounds' interaction with biological membranes and improve their binding affinity to target proteins compared to non-prenylated counterparts. Prenylated and geranylated chalcones form a significant subclass of flavonoids, abundantly found in nature. These compounds exhibit a wide range of biological properties, including antibacterial, antimalarial, antifungal, antidiabetic, antitumor, antioxidant, anti-inflammatory, and enzyme-inhibitory activities. The α,β-unsaturated carbonyl system, along with the presence of aromatic rings on either side of the carbonyl group, and a side chain confirms that compound T4 is a geranylated chalcone, a derivative of flavonoids. These structural features contribute significantly to the compound’s biological efficacy [7].

4. Conclusion

The Column chromatographic separation and purification from Ethylacetate fraction led to the isolation of compound 2’,6’,4-trihydroxy-3’-methoxy-4-O-prenyloxy chalcone a geranylated chalcone. This revealed the plant is a rich source of secondary metabolites and this compound was reported for the first time from *Terminalia brownii* and contribute to the taxonomy of the plant.

Abbreviations

UV	Ultraviolent

IR	Infra-red
FTIR	Fourtier
2D	Two Dimension
NMR	Nuclear Magnetic Resonance
DCM	Dichloromethane
EAF	Ethylacetate Fraction
HEX	Hexane
TLC	Thin Layer Chromatography
SAR	Structural Activity Relationship
PPM	Part per Million
ABU	Ahmadu Bello University
T.brownii	*Terminalia brownii*
Tb	*Terminalia brownii*

Authors Contributions

Tijani Tawakaltu Omolara: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - original draft, Writing - review & editing

Akande Amatulhafeez: Conceptualization, Writing - review & editing

Olaiya Akeem Ayodele: Conceptualization, Formal Analysis, Project administration, Writing - review & editing

Dauda Garba: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing - review & editing

Atiku Ibrahim: Supervision

Muhammed Ibrahim Sule: Supervision

Conflicts of Interest

There was no conflict of interest beteween the authors during the project.

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