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Isolation and Characterization of Bioactive Compounds from *Trigoniulus corallinus* (Red Millipede)

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Abstract

This study investigated the zoochemicals derived from *Trigoniulus corallines* (red millipedes). Three different solvents—n-hexane, chloroform, and methanol—were employed for extraction. The methanol extract yielded the highest percentage at 5.6%, followed by chloroform (2.2%) and n-hexane (0.8%). Preliminary zoochemical screening revealed the presence of terpenoids, phenols, sterols, and flavonoids in all extracts, with saponins exclusively detected in the methanol extract. Chromatographic separation of the methanol extract, chosen due to its superior antibacterial activity, led to the isolation of a pale-yellow compound coded YB. Physicochemical characterization indicated that compound YB is a pale yellow crystalline solid with a melting point of 58°C, and is soluble in chloroform, ethyl acetate, methanol, and n-hexane. Spectroscopic analyses, including Fourier Transform Infrared (FTIR), Ultraviolet-visible (UV-Vis), 1H Nuclear Magnetic Resonance (NMR), and 13C NMR, were utilized to elucidate the structure of compound YB. Based on these spectroscopic data and comparison with existing literature, the structure of compound YB was confirmed as 1,3-Diphenylprop-2-en-1-one, commonly known as Chalcone. These findings suggest that red millipede extracts, particularly the methanol extract and its isolated compounds like chalcone, represent a promising source of antimicrobial agents for future research and development.

Keywords: Characterization, Isolation, Millipede, Zoochemical.

Introduction

A pressing global health challenge is the escalating issue of antimicrobial resistance in microbial infections, necessitating the urgent discovery of novel and alternative antimicrobial treatments as the efficacy of existing antibiotics declines [1]. Natural products offer a promising avenue in this critical endeavor, presenting numerous potential advantages in combating antibiotic resistance. These valuable compounds can be sourced from a diverse range of organisms, including plants, animals, and microbes. Historically, natural compounds have been

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recognized for their inherent antibacterial properties, a characteristic that remains crucial in contemporary medication research programs [2].

Among various natural sources, millipedes are known to produce naturally occurring antibacterial substances. Millipedes belong to the class Diplopoda, which represents the third most diverse group of terrestrial arthropods, encompassing approximately 12,000 formally recognized species. A distinctive physiological feature of Diplopoda is the presence of a pair of exocrine defense glands. These glands are responsible for synthesizing and secreting a variety of volatile substances, including but not limited to alkaloids, quinones, phenols, and cyanogenic compounds, which contribute to their defensive mechanisms and, potentially, their antimicrobial properties. This study aims to investigate the chemical composition, antimicrobial activities, and characterize bioactive compounds derived from red millipedes (*Trigoniulus corallinus*), contributing to the search for new antimicrobial agents [3].



Plate 1: Picture of red Millipede

Methodology

Sample Collection and Preparation

Red millipedes were collected from Garugga, Babban Runji Area, Yabo Local Government, Sokoto State, Nigeria (12° 42' 05"N, 5° 00' 45"E) between July and September 2024. The specimens were identified at the Department of Zoology, Usmanu Danfodiyo University, Sokoto. After washing, the samples were dried at 40°C, ground into powder, and stored in airtight containers prior to extraction.

Extraction Process

A four hundred grams (400 g) of millipede powder was macerated with 1.5 L of n-Hexane for 72 hours, with occasional stirring. The solvent was filtered using Whatman-1 filter paper, and the filtrate was air-dried to obtain the n-Hexane extract. The residue was sequentially extracted with chloroform and methanol using the same procedure. The percentage yield was calculated as follows:

$$\% \text{ yield } = \frac{\text{weight of extract}}{\text{sample weight}} X\ 100$$

Zoochemical Screening

Chemical tests were conducted to detect secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, and phenols, following established protocols [4].

Chromatographic Technique

The methanol extracts was subjected to Column Chromatography to isolate bioactive compound responsible for its antibacterial activity. The methanol extract was selected because it demonstrated the highest antibacterial activity in the antimicrobial studies.

Tin Layer Chromatographic Analysis

As stated by Umar [4]and Yusuf [5] , One -way ascending thin-layer chromatography was used to analyze all of the extracts on silica gel 60 F_{254} pre-coated aluminium sheets. Using scissors, the plates was cut, and a pencil mark was be made around 1 centimeter from the plate's bottom. After slightly dissolving the extract in the suitable solvent, the dissolved extract was applied to the plates using capillary tubes and left to dry. The various solvent systems were used in a chromatographic tank to produced chromatograms which was allowed to air dry and observed in ambient light, under ultraviolet light (254 and 365 nm), and after being sprayed with 10% sulfuric acid and heated in an oven for 5 to 10 minutes at 105 ^{o}C. Each component's Retention factor (R_f) was determined using the formular in equation as follows:

$$R_f = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by solvent}}$$

Column Chromatography

A 50 cm x 5 cm, 500 cm^3 glass column and a stationary phase silica gel of 60-120 mesh size were used. Wet packing method was used for silica gel column chromatography. Methanol extract (6g) was subjected to column chromatography using gradient elution technique using mixture of Hexane: ethyl acetate 8:2, ethyl acetate methanol 9:1, methanol: water 9:1, EA total of 22 collections of 20 mL each were made and combined based on their TLC to afford 3 major fractions. The ethyl acetate: Methanol Fractions gave better TLC spots therefore it was air dried and coded as “YB”.

Characterization of YB

Melting Point Determination

The compound YB (10 mg) was grinded into fine powered and place small amount into a capillary tube and was placed in melting point machine. The melting point machine was set 15 ^{0} C and was slowly increased and observed.

Solubility test

The grinded YB compound (10 mg) was inserted in a test tube and10 mL of solvents were added differently (Methanol, ethyl acetate, n-hexane and Chloroform) shake gently for few minutes and observed.

Spectral analysis

FTIR, UV-VIS and 1D-NMR were used to elucidate the structure of the compound. YB

Results

Extraction Yields

The methanol extract yielded the highest percentage (5.6%), followed by chloroform (2.2%) and n-Hexane (0.8%) the percentage yield is presented in Table 1.1

Table 1.1: The percentage yield of the extracts (%)

Solvent	Weight(g)	Yield (%)
n-Hexane	3.2	0.8
Chloroform	8.8	2.2
Methanol	22.4	5.6

Zoochemical Composition

Terpenoids, phenols, sterols, and flavonoids were present in all extracts, while saponins were detected only in the methanol extract. The zoochemical composition of red millipedes is presented in Table 1.2

Table 1.2: Zoochemical Screening of red millipede

Constituents	Test	N-Hexane Extract	Chloroform Extract	Methanol Extract
Saponins	Frothing	-	-	+
Terpenoids	Libermen-Burchard's	+	+	+
Phenols	Ferric chloride	+	+	+
Sterols	Salkowkis's	+	+	+
Flavonoids	Ferric Chloride	+	+	+

Key: (+) = Detected, (-) = Not Detected

Chromatographic Separation

Column Chromatography

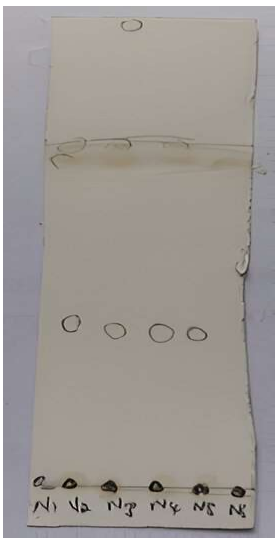
The result of Column Chromatography from methanol extract of red millipede carried out using different solvent combinations afforded five major fractions as presented in Table 1.3.

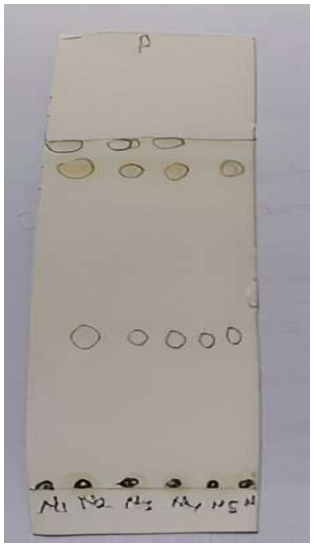
Table 1.3 Column Chromatographic collections from methanol extracts

S/No	Solvent system	No of eluates (20 mL each)	Code
1	Ethyl acetate: N-Hexane(8:2)	1-6	O
2	Ethyl acetate: Methanol (9:1)	7-12	P
3	Methanol : water (9:11)	13-17	Q
4	Ethyl acetate: N- Hexane (7:3)	18-22	R

5	Ethyl acetate : N-Hexane : water(7:2:1)	23-27	S

The TLC profile of Column collections from methanol extract conducted using chloroform: ethyl acetate as solvent system revealed various spots after spraying with 10% H_2SO_4 followed by heating at 105 ^{0}C.





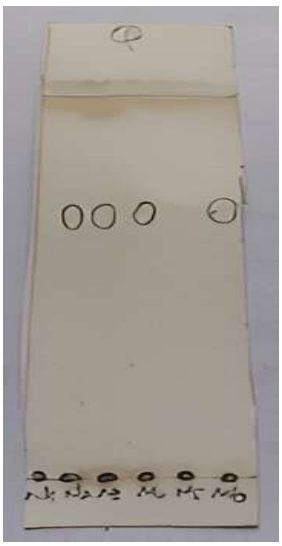


Plate I: EA: Cl (8:2) II: EA:Cl (7:3) III: EA:Cl (1:1)

Key: EA= Ethyl acetate, Cl= Chloroform

Isolation of Compound YB: Compound YB gave a single homogenous spot on TLC using ethyl acetate n-hexane (8:2) and the result is presented in plate 1V-VI and Table 1.4.



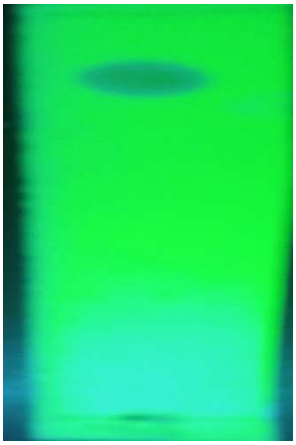




Plate IV Plate V Plate VI

Table 1.4 : R_F Value of YB

Solvent System	No spot	Colour of spot after spraying with 10% H_2SO_4	R_F Value
EA: Nh	1	Pale yellow	0.61

Table 1.5: Physiochemical Parameter of Compound YB

Solubility	**Physical Appearance**	**Melting Point**
Chloroform, ethyl acetate, methanol and n-hexane	Pale yellow crystalline solid	58 ^{0}C

Spectroscopic Analyses

Fourier Transform Infrared Spectroscopy of YB

The FTIR Spectrum of compound YB is presented in figure 1 and result is presented in Table 1.6.

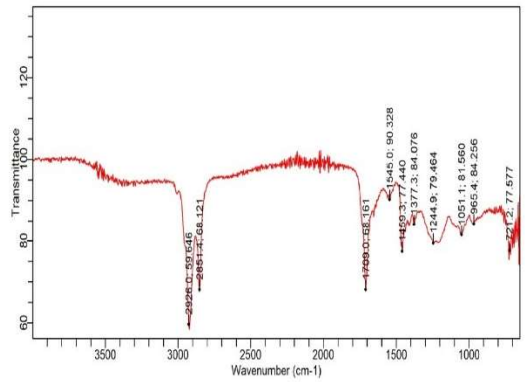


Figure 1: FTIR Spectrum of YB

Table 1.6 Infrared Absorption of Compound YB

Band	**YB**	**Literature(Shingare *et al*., 2024)** [6]
C = O	1709	1654.9
C = C (Ar)	1545	1589.4-1600
C=C (Alkene/alkane)	1450.5	1500
C-H	3020	3020-3110

The Fourier Transform Infrared (FTIR) spectrum of the YB indicated absorption at 2926 cm-1 possibly due to C-H stretching vibrations (alkanes), 1709 cm-1 correspond to C=O stretching (carbonyl groups), 1545 cm-1 is indicative of C=C bending (alkenes), and 1450.5 cm-1 C=C bending (aromatic): is related to and Peaks below 1000 cm-1 is a finger print region the FTIR data corresponds to Kendeson [7].

Ultraviolet–visible spectroscopy (UV-Vis) Spectroscopy of YB

The UV-VIS Spectrum of compound YB is presented in figure 2 and result is presented in Table 1.7

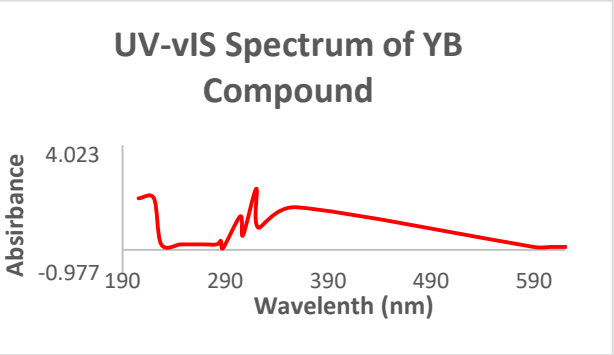


Figure 2: Ultraviolet–visible spectroscopy (UV-Vis) of compound YB

Table 1.8: Ultraviolet visible Absorption of YB Compound

Wavelength (λmax) (nm) – YB	**Literature(Jacob *et al.*, 2020** [8]	**Electronic Transition / Chromophore**
227.5	220 – 260	π→π*
242	220 – 260	π→π*
285.5	285.6	π→π*
320	300 – 340	π→π*
Other peaks (<220 nm)	(Below 220 nm)	π→π*

Ultraviolet–visible spectroscopy (UV-Vis) spectrum of the YB (Figure 13) indicated absorbances 2.189 at 205.5 nm and 220.5 nm wavelengths which are typical of π-π* transitions, often seen in aromatic compounds or conjugated systems. The strong absorbance suggests the presence of a benzene ring or similar aromatic structures. Absorbance of 2.596 at 320 nm is significant peak, indicating strong electronic transitions. Compounds with extended conjugation or specific functional groups like carbonyls (e.g., ketones, aldehydes) can show absorbance in this range. Absorbance of 1.813 at 359 nm corresponds to another electronic transition associated with conjugated systems or possibly some complex chromophore. Lower Absorbance Values at 227.5 nm to 287.5 nm wavelengths indicates minimal absorption, suggesting less conjugation or possibly the presence of saturated or aliphatic compounds. The UV-Vis data corresponds to Shingare *et al.,* 2024 [6].

Nuclear Magnetic Resonance (NMR) Spectroscopy of YB

^{1}H NMR of YB

The ^{1}H-NMR spectrum of YB is presented in figure 14

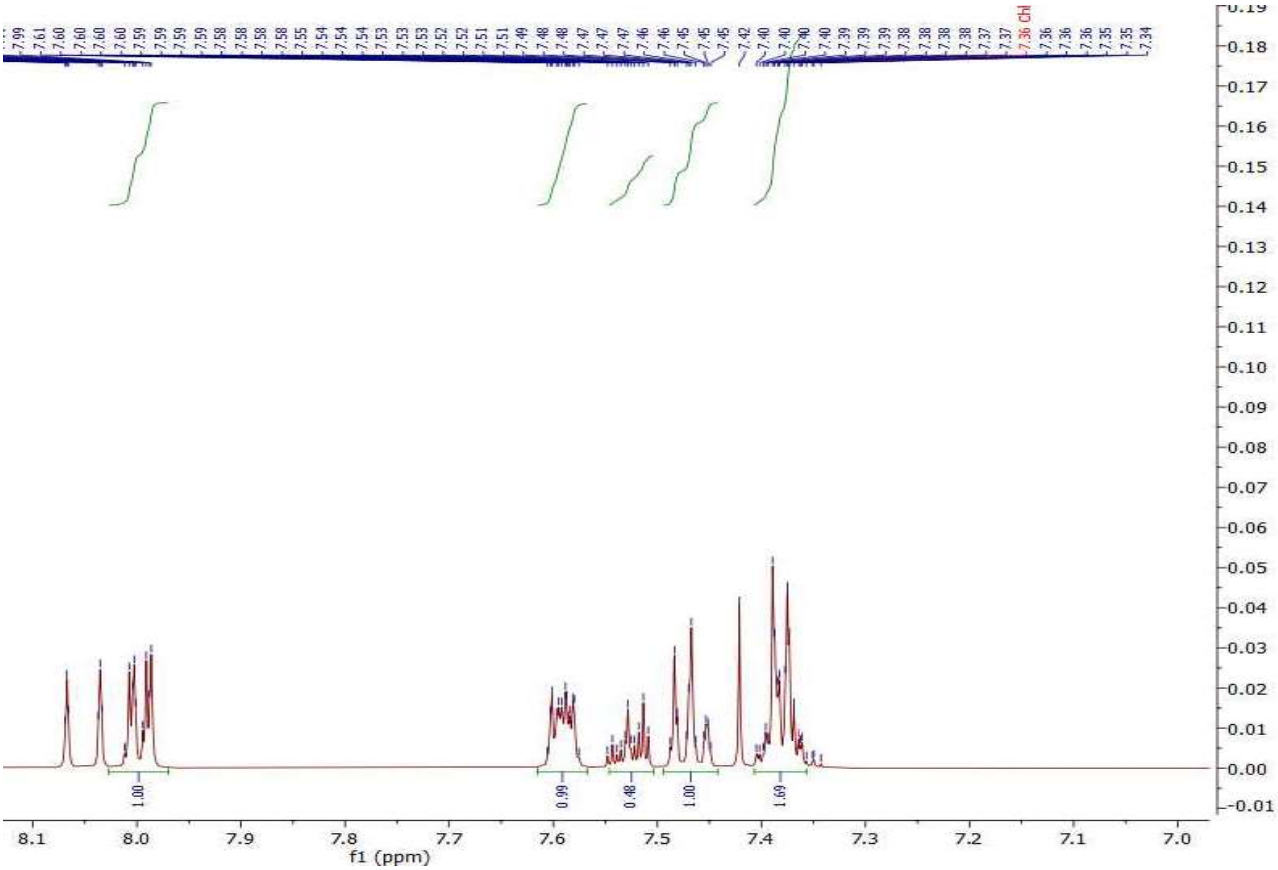


Figure 2: ^{1}H-NMR of the compound YB

^{1}H NMR (400 MHZ) of compound YB revealed signals at δ 7.99-8.02 (d, 1H, J = 15.72 Hz, Hβ), 7.63 – 7.56(m, 3H, Ar), 7.45 – 7.48(d, 1H, J = 15.73 Hz, Hα), 7.32 – 7.40 ((m, 5H, Ph-H), 7.9 – 8.0 (m, 2H, Ph-H

^{13C} NMR of YB

The ^{13}C NMR spectrum of YB is presented in figure 15

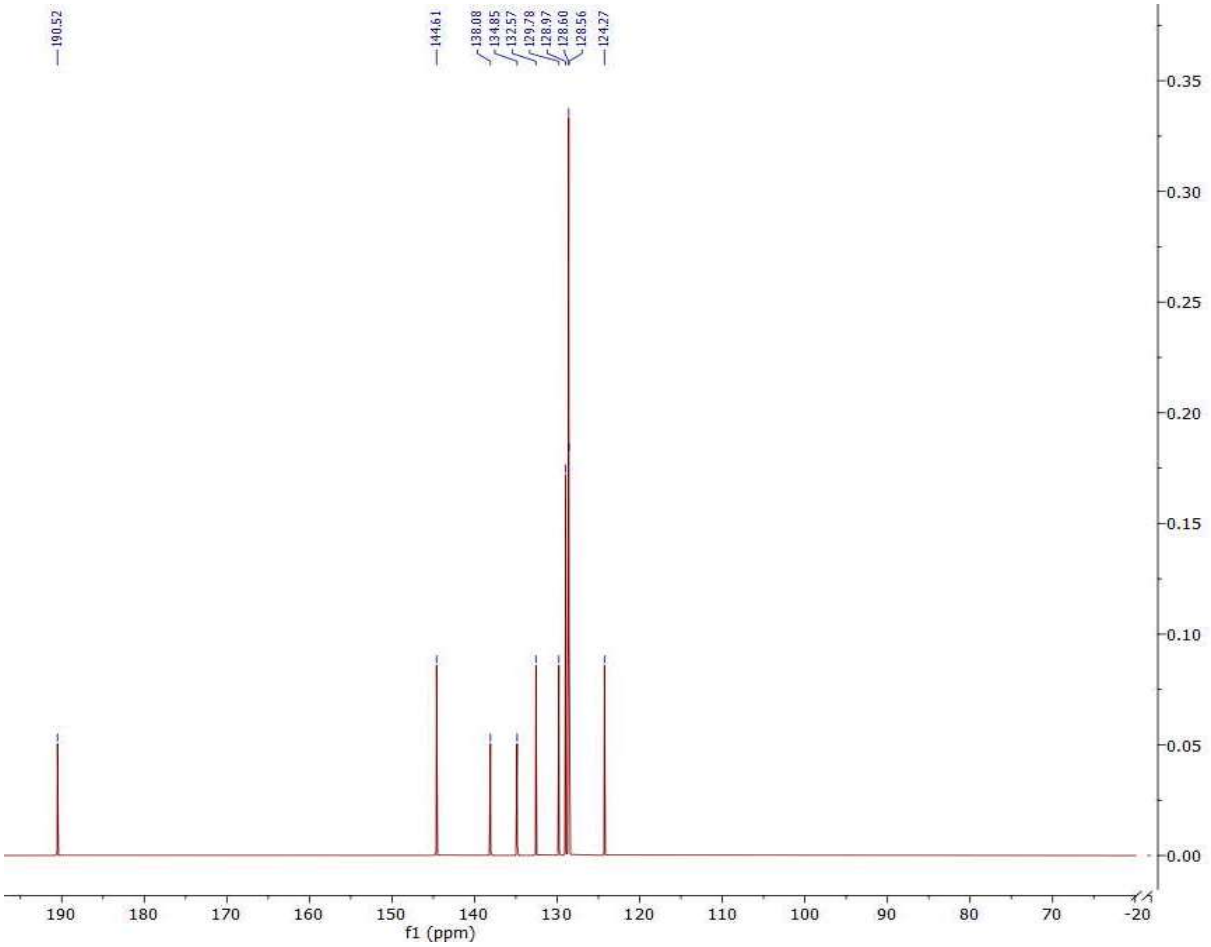


Figure 3: ^{13}C NMR of Compound YB

^{13}C NMR (100 MHZ) spectrum of YB revealed signals at δ 191.57, 144.61, 138.08, 134.85, 132.57, 129.78, 128.97, 128.60, 128.56, 124.27

Table 1.9 NMR spectra data of YB and comparison with literature

^{1}H NMR (YB) δ (ppm)	**^{1}H NMR Ritter et al. (2015)**	**^{13}C NMR(YB)**	**^{13}C NMR Ritter et al. (2015)**
7.99-8.02 (d, 1H, J = 15.72 Hz, Hβ)	7.79 (d, 1H, J = 15.72 Hz, Hβ)	191.57 Carbonyl (C=O)	189.95 (Carbonyl, C=O)
7.63 – 7.56(m, 3H, Ar)	7.54 – 7.60 (m, 3H, Ar)	138.08 Alpha carbon (C-2	137.73 Alpha carbon (C-2
7.45 – 7.48(d, 1H, J = 15.73 Hz, Hα)	7.50 (d, 1H, J = 15.73 Hz, Hα)	144.61 beta carbon (C-3)	144.30 beta Carbon C-3)
7.32 – 7.40 ((m, 5H, Ph-H)	7.36-7.38 (m, 3H, Ph-H)	134.85 and 132.57 quaternary carbon (C-4 & 10)	134.40 and 132.30 (quaternary)
7.9 – 8.0 (m, 2H, Ph-H)	8.00 – 8.09 (m, 2H, Ph-H)		
		129.78 Aromatic carbon (C-6,9)	128.03 (Aromatic C-)
		128.97 Aromatic carbon (C-7)	128.15 (Aromatic C-)
		128.60 Aromatic carbon (C-8) and very similar 13,15	128.03 (Aromatic C-)
		128.56 Aromatic carbon (C-12& 14)	127.98 (Aromatic C-)
		124.27 Aromatic carbon (C-11)	124.36 Aromatic carbon (C-11)

Isolation and structural elucidation of YB

Chromatographic separations

Chromatographic separation of Methanol extract being most active in the antimicrobial studies led to isolation of pale-yellow compound coded YB with a single clear homogenous spot on TLC plate and R*f* Value of 0.6, melting point of 58^{0}C soluble in methanol, ethyl acetate and Chloroform. The colour and solubility observed corresponds to Shingare *et al*. [6] that reported that natural occurring chalcones are generally crystalline solid and poses different color as brown, yellow, orange and are soluble in organic solvents. The Rf value and melting point corresponds to Jacob *et al*.[8] that reported that of 1, 3-Diphenylprop-2-en-1-one has Rf value of 0.69, melting point of 48^{0}C.

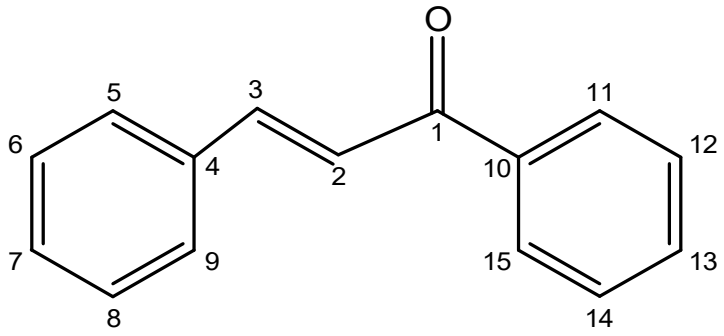
FTIR and UV-Vis Spectroscopy

The Fourier Transform Infrared (FTIR) spectrum of 1,3-Diphenylprop-2-en-1-one shows characteristic peaks of aromatic hydrocarbons (C–H stretches near 3050 cm^{-1} and ring vibrations at 1600–1450 cm^{-1}). A strong peak observed around **1680–1660 cm^{-1}** confirms the presence of the conjugated carbonyl group (C=O), which is shifted to a lower wavenumber due to resonance with the adjacent alkene and phenyl rings. The fingerprint region (1500–650 cm^{-1}) reveals alkene (C=C) bending and aromatic substitution patterns, consistent with the reported structure.

The UV-Vis spectrum indicates that the compound YB has a conjugated system with significant absorbance in the UV region due to π → π* transitions. The strong peaks at 205.50 nm and 220.50 nm are characteristic of aromatic while the peaks around 300–350 nm suggest extended conjugation, possibly involving carbonyl groups. The compound does not absorb significantly in the visible region.

NMR Spectroscopy

The compound YB was obtained as pale yellow compound, ^{1}H-NMR and ^{13}C- NMR show chemical shift values typhical of flavonoids. The H-NMR spectrum of compound indicated the presence of 1,3-Diphenylprop-2-en-1-one via ^{1}H NMR (400 MHZ) of compound YB revealed signals at δ 7.99–8.02 (d, 1H, J = 15.72 Hz) is Hβ vinylic proton, β-position due carbonyl deshielding, δ 7.63–7.56 (m, 3H, Ar-H) is Ho (ortho protons on B-ring, ketone side due electron withdrawing effect, δ 7.45–7.48 (d, 1H, J = 15.72 Hz) is Hα (vinylic proton, α-position due conjugation of Ph δ 7.32–7.40 (m, 5H, Ph-H) is Hm/Hp (meta/para protons on A-ring & 1 B-ring proton due to no direct effect of C=O, 5. δ 7.9–8.0 (m, 2H, Ph-H) is Ho' (remaining ortho protons on B-ring, ketone side due the proximity of C=O which is good agreement with Ritter et. Al., 2015 [9]. ^{13}C NMR (100 MHZ) spectrum of YB revealed signals at δ 191.57 is Carbonyl (C=O), δ 144.61 is β-Carbon (vinylic, between rings), δ 138.08 is α-Carbon (vinylic, adjacent to C=O), δ δ134.85 is Quaternary aromatic (ring A junction), δ 132.57 is Quaternary aromatic (ring B junction), δ 129.78, δ 128.97 are Aromatic (ring A) δ 128.60, δ 128.56, δ 124.27 are Aromatic (ring B) based on the FTIR, IR and NMR data of YB and Comparison with exiting data in the literature[6,8,9] the structure of YB was confirmed to be Chalcone.



Chemical Structure of **1,3-Diphenylprop-2-en-1-one**

The mechanism by which the extract elicits it antibacterial effect might be due to either

presence of secondary metabolites or via inhibition of antibacterial growth by interfering with the production of bacterial protein and DNA replication.[10] Chalcone as one of the bioactive constituents of red millipedes has been considered as potential candidate Because chalcone derivatives have a ketoethylenic moiety (CO−CH=CH−) they are valued species. Chalcones and their derivatives have a broad range of antiproliferative, antifungal, antibacterial, antiviral, antileishmanial, and antimalarial pharmacological activities because of the presence of a reactive α,β-unsaturated carbonyl group[11]

Conclusion

The study revealed that the in the extraction of red millipedes methanol extract yielded the highest percentage, and zoochemical screening detected terpenoids, phenols, sterols and flavonoids present in methanol, n-hexane and Chloroform extract, while saponins were detected in methanol extracts only. Being methanol extract with most effective activity and yielded the highest percentage of extract and it was subjected to Chromatographic studies which led to the isolation of a pale-yellow compound coded YB. The spectroscopic analyses confirmed the structure of isolated compound to be 1,3-Diphenylprop-2-en-1-one.

Recommendations

1. The isolated chalcone compound (YB) should be subjected to broad-spectrum antimicrobial assays, including activity against multidrug-resistant (MDR) pathogens, to confirm its therapeutic potential.

2. Preclinical studies, including toxicity tests and pharmacokinetic profiling, are recommended to assess the safety and efficacy of the compound for potential pharmaceutical applications.

3. Derivatives of 1,3-Diphenylprop-2-en-1-one can be synthesized to explore structure-activity relationships (SAR) and potentially enhance antimicrobial potency.

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