

Isolation and Identification of Chemical Constituents from Hydro-Ethanol extract of *Terminalia schimperiana* Root by TLC and Gas Chromatography-Mass Spectrometry

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Abstract

In this study, identification and isolation of the chemical compositions of hydro-ethanol extract of *Terminalia schimperiana* root by TLC and Gas chromatography-mass spectrometry was carried out. The powder sample was extracted with ethanol and water by cold maceration, the ethanol was evaporated from the extract using rotary evaporator to obtain semi solid masses and dried. Qualitative analyses of hydro-Ethanol extract of *Terminalia schimperiana* by using GC- MS showed the presence of compounds like n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecanoic acid (Z,Z), Ethyl 9.Cis.,11.trans-Octadecadienoate and Tris (cyclopentadienyl-cobalt)-hexapropenylbenzene. The compounds detected might be responsible for the reported therapeutic activities of the plant extract.

Keywords: *Terminalia schimperiana*; Thin layer chromatography (TLC); Gas chromatography-mass spectrometry (GC-MS); Hydro-ethanol.

Introduction

Researchers have developed increased interest for the preparation of novel drugs or Incremental innovative drugs derived from herbal plants through the isolation and identification of some bioactive compounds which might be useful for disease therapy comparable with synthetic drugs that might be laden with adverse effects. *Terminalia schimperiana* is an important medicinal plant. It is a broadleaved small tree with tiny flowers that form pale spikes at the base of the leaves. It can be found in open forest habitats with more than 1300 mm of rainfall per year and can reach up to 7–14 m. In the closed forest, it is a dominant tree species that forms typically part of the forest canopy (Jones, 1993). It belongs to the order: myrtales, family: Combretaceae, genus: *Terminalia* and species: *T. schimperiana*, it is known as Idi in Yoruba language (Arbonnier, 2004). This plant species is a widely distributed one in the tropical regions of the world, it stretches from Guinea, to Ethiopia, to Democratic republic of Congo, to Uganda and to Northwestern Tanzania. In Africa, the twigs may be chewed for oral hygiene and the bark is applied to wounds for healing. Laboratory, experiments found *in vitro* antibiotic properties against *Staphylococcus* (Batawila, 2005) and found antifungal properties *in vitro* (Wagner, 1996). *Terminalia schimperiana* plant root has also been claimed to contain some important bioactive compounds that can produce erectogenic and aphrodisiac activity as reported by the locals. Therefore, the aim of this work was to investigate and isolate the chemical constituents in the hydro-ethanolic extracts of *Terminalia schimperiana* root by using thin layer chromatography (TLC) and Gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Plant Material

Terminalia schimperiana root was collected from a local government area of Kwara state in Nigeria. The Identification and authentication of plant was carried out at the botany unit of the Department of Pure and Applied Biology, Ladoke Akintola College of science and technology, Ogbomoso, Nigeria.

Chemicals

All chemicals like butanol, hexane, chloroform, methanol etc. used were of analytical grade and were purchased from Sigma Chemical Co.

Sample Preparation

The root was cleaned, cut into pieces and dried, then the dried pieces were pulverized into coarse powder using an electric grinder.

Hydro-Ethanol Extract Preparation

130g of the coarse powder was subjected to maceration in 700ml of 70% (v/v) hydro-ethanol with constant shaking for 120 hrs at room temperature. The extract was filtered with Marcelin cloth and the filtrate was further filtered with Whatman no. 1 filter paper. The filtrate was concentrated using rotatory vacuum evaporator till all the ethanol has been removed and the remaining extract was concentrated on water bath after which the percentage yield was calculated and preliminary phytochemical tests were carried out on the extract.

TLC Finger printing of 2° metabolites present in hydro-ethanol fraction

After phytochemical screening, the prepared hydro-ethanol fraction was subjected to thin layer chromatography analysis using standard methods described by (5). 10ul of sample was applied to aluminum coated silica gel 60 GF 254 TLC plate and n-butanol: GAA: water (4: 1: 1) was used as the mobile phase of tank with saturation time of 30 min, the hydro-ethanol spots were identified using ferric chloride (detecting Reagent) and under 254 & 366 NM in a TLC visualizer (CAMAG) Solvent

GC-MS analysis

The GC-MS analysis of the plant extracts from the root of *Terminalia schimperiana* was carried out to determine the compounds present. Perkin Elmer Clarus 600 GC system with a fused silica gel column (30 mm, 0.25mm ID, film thickness 0.25 μ m) coupled with a Perkin Elmer Clarus 600C MS was used for the analysis. Data or spectra was detected by an electron ionization system with ionization energy of 70 eV. Inert helium gas (99.999%) was used as a carrier gas at a constant flow rate of ± 1 mL/min. Mass transfer line and injector temperatures were at 220 and 290 °C, respectively. The extract sample was diluted with methanol (1/100, v/v, in methanol). The tested samples were filtered with 0.45 μ m Millex membrane filter paper (Millipore, France) to remove any dust particles. One microliter filtered test sample was injected in the split mode. The split ratio was 120:1. The percentage (%) of the extracts constituents from the *Terminalia schimperiana* was expressed as percentage by peak area. The whole process was carried out carefully from the light and heat. Bioactive compounds in the plant extracts of *Terminalia schimperiana* were identified based on GC retention time. The mass spectra were matched with computer matching with those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems).

Result and Discussion

Physical property of the extract

The physical appearance of hydro ethanol extracts from *Terminalia schimperiana* was dark brown in colour, sticky and sweet smell.

Chemical composition of hydro ethanol extract

The various secondary metabolites was identified in the hydro ethanol fractions of *Terminalia schimperiana* using thin layer chromatography analysis and the RF of the prominent spots were identified as shown in table 2 and in figure 1.

The hydro ethanol extract was analysed using GC-MS and was found to contain 5 bioactive compounds (figure 2 and table 3). The analysis of the extract showed presence of compounds like n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecanoic acid (Z,Z), Ethyl 9.Cis.,

11.trans-Octadecadienoate, Tris(cyclopentadienyl-cobalt)-hexapropenylbenzene. A complete analysis report is given in table 3 below.

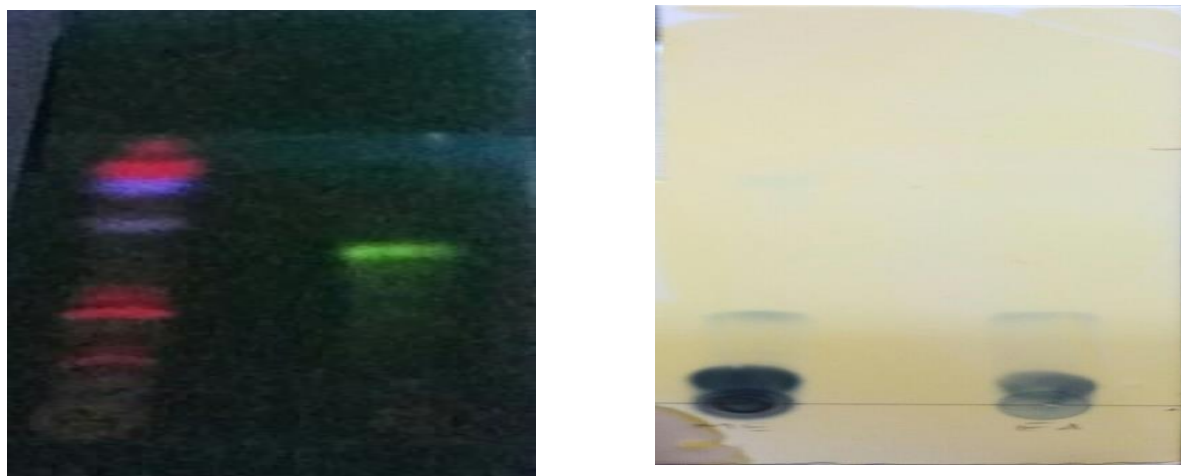


Figure 1: TLC finger print of Hydro-ethanol fraction and Saponin fraction of *Terminalia schimperiana* root showing five spots. (a) UV view (b) View with FeCl reagent

Table 1: Showing the yield and physical property of *Terminalia schimperiana* root extract obtained

S/No.	Name of solvent	Colour	Consistency	Yield (%w/w)
1	Hydro-Ethanol	Dark brown	Sticky	10.62

Table 2: TLC Finger Print of hydro-ethanol fraction of *Terminalia schimperiana* root

S/N	Sample spot	Rf spots
1	Hydro alcohol fraction	0.37, 0.51, 0.79, 0.82, 0.86

Table 3: Chemical composition of hydro ethanol extract of *Terminalia schimperiana* root

S/No	Retention time	Name	% Area	Molecular Formular
1	14.5	n-Hexadecanoic acid	9.60	C ₁₆ H ₃₂ O ₂
2	14.32	Hexadecanoic acid,ethyl ester	9.85	C ₁₈ H ₃₆ O ₂
3	15.28	9,12-Octadecanoic acid (Z,Z)	11.75	C ₁₈ H ₃₂ O ₂
4	15.40	Ethyl 9.Cis.,11.trans-Octadecadienoate	5.24	C ₂₀ H ₃₆ O ₂
5	24.57	Tris(cyclopentadienyl-cobalt)-hexapropenylbenzene	29.03	C ₃₉ H ₄₅ Co ₃

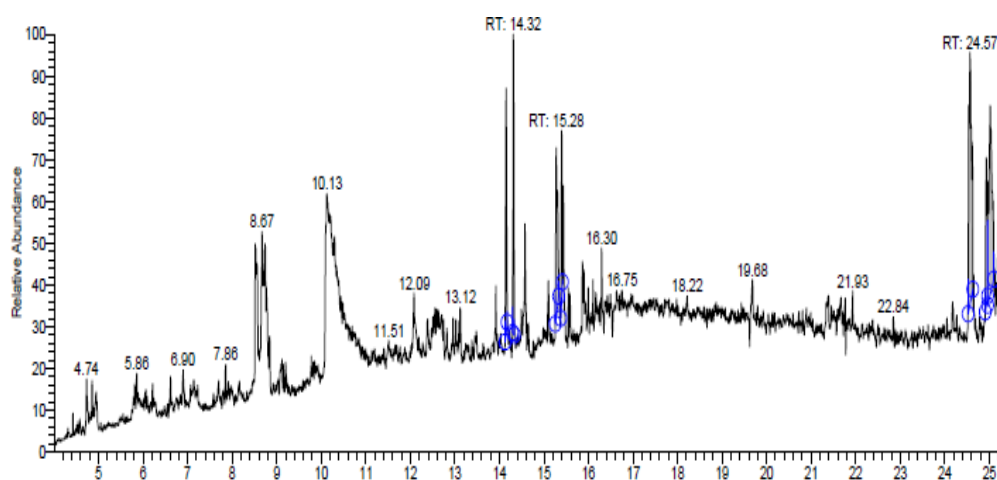


Figure 2: GC-MS Finger print of Hydro-ethanol fraction of *Terminalia schimperiana* root

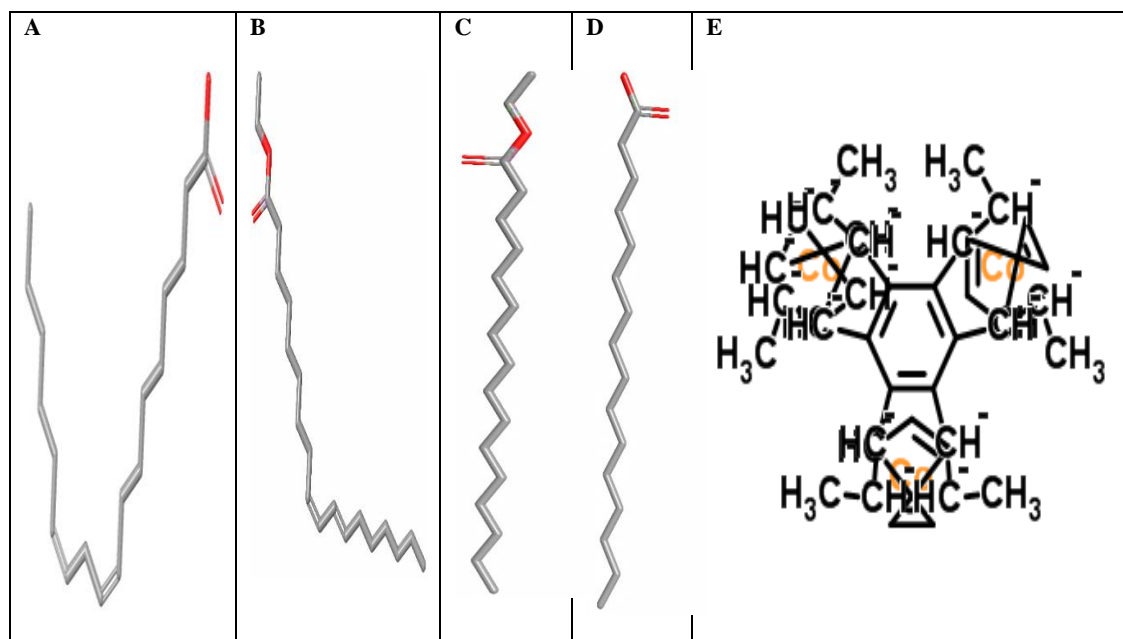


Figure 3: Structures of the hydro ethanol components of *Terminalia schimperiana* root

(a). n-Hexadecanoic acid (b). Hexadecanoic acid, ethyl ester (c). 9,12-Octadecanoic acid (Z,Z)
 (d). Ethyl 9-Cis., 11 trans-Octadecadienoate (e). Tris (cyclopentadienyl-cobalt)-hexapropenylbenzene

The reported medicinal activities of *Terminalia schimperiana* is enormous. The various compounds that have been identified from the hydro ethanol extract probably indicate that the plant bioactive components could be effective as a potent therapeutic medicinal plant either by working together or working as individual bioactive component. The compounds available might be responsible for the reported therapeutic activities of the plant such as aphrodisiac, antioxidant, antimicrobial, anti-fungal and cytotoxicity activities.

Conclusion

The 5 bioactive components isolated from the hydro ethanol extract of *Terminalia schimperiana* root might be responsible for the Pharmacological activities often experienced by the locals that use it. Therefore, the identification of the bioactive components of *Terminalia schimperiana* by GC-MS was pharmacologically relevant.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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