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Research Article

Phytochemical Analyses of *Terminalia schimperiana* (Combretaceae) Root Bark Extract to Isolate Stigmasterol

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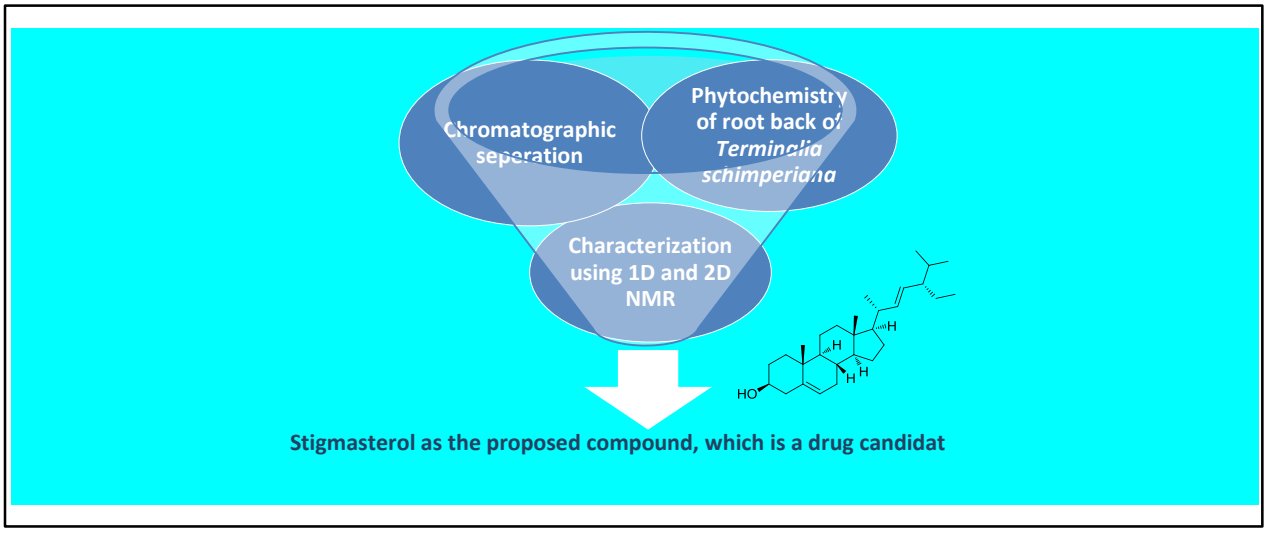
ABSTRACT

Phytochemical screening of *Terminalia schimperiana* (Combretaceae) Root Bark showed presence of flavonoids, tannins, steroid carbohydrates and terpenoides in *n*-hexane, ethylacetate and methanol as solvents of extraction. Isolated stigmasterol from *Terminalia schimperiana* was a white-yellow crystal which characterized using ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC and HMBC spectral techniques. Research studies clearly revealed that *Terminalia schimperiana* root bark extract has potential to be exploited in the pharmaceutical firm in the search for stigmasterol related drug from nature.

GRAPHICAL ABSTRACT



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Introduction

Medicinal plants are important parts of our nature. They serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines [1,2]. Our ancestors used natural substances they considered to have medicinal value to ease their sufferings caused by acute and chronic illnesses, physical discomforts, wounds and injuries, including terminal illnesses [3]. Since ancient times, plants with therapeutic properties have secured an important place in the healing practices and treatment of diseases [4-6]. Plants still remain the basis for development of modern drugs and medicinal plants have been used for decades in daily life to treat diseases all over the world.

This research work is focused on indigenous natural herbs that have curative properties, because traditional medicine could be a better/affordable treatment than the currently used drugs [7]. The justification for this study is based on the wide medicinal applications of this plant in general. It is clear that the plant can be further screened to provide a basis for not only further investigation but also its continued use in ethno-medicine [8].

Terminalia schimperiana is a broadleaved small tree that can reach up to 7–14 m, variably deciduous in the dry season to semi-evergreen, depending on the climate. The leaves are alternate, simple, and elliptic to obovate, 9–15 cm long and 3–8 cm broad, green above with pale undersides. The flowers are tiny and form pale spikes at the base of the leaves. The fruit is a samara with a single wing 6–9 cm long, that turns brown with age [9]. It can be found in open forest habitats with more than 1300 mm of rainfall per year, when it is found in closed forest, it typically part of the forest canopy and it may be the dominant tree species where it is found [10,11].

It belongs to the family *combretaceae*, commonly called the 'Tuit plant", it is known as "Kwuegh", in Tiv language, "Buashe" in Hausa language, and "Idi" in Yoruba language.

In most parts of West Africa, *T. schimperiana* is used as a medicinal plant [12]. The bark is used to treat wounds; the twigs may be chewed to promote oral hygiene, dental care, as laxative and catarrh. Pulverized roots and root bark applied to wounds, burns, ulcer and skin diseases including leprosy. Root powder is taken to treat epilepsy; root decoctions are used to treat malaria, hepatitis. In laboratory, experiments on extracts of the plant were found to have *in vitro* antibiotic properties against staphylococcus [7] and the plant extract has been found to also have antifungal properties *in vitro* [13] and the leaves extract also was reported to reduce blood glucose level in albino rats [14] among the 'Tiv' people of central Nigeria, its roots are boiled and administered orally to treat diabetes [15].

Materials and methods

Root bark of *T. schimperiana* was collected from the wild near Tse-Mtswenem, Mbyayigha of Tarka Local Government Area of Benue State Nigeria and was authenticated at the College of Forestry and Fisheries, Federal University of Agriculture Makurdi, Benue State, Nigeria with a Voucher No FHI/0259 which was deposited at the College herbarium. The root bark was washed in clean water to remove dirt, air dried at room temperature for three weeks, pulverized with the aid of pestle and mortar into coarse powder. It was stored in air tight container until required for the experiment [14-16].

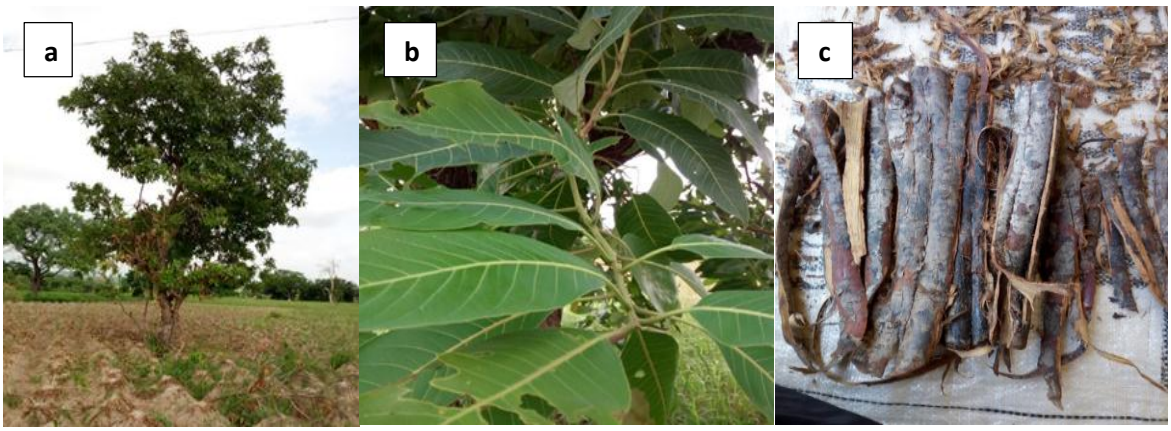


Figure1. (a) *Terminalia schimperiana* Plant. (b) *T. schimperiana* Leaves, (c) Root back of *Terminalia schimperiana* (part worked on)

Extraction

Dried, pulverized root bark of *T. schimperiana* (500 g) was successively extracted *via* maceration with hexane, ethyl acetate and methanol. The mixture was filtered with Whatman filter paper No.1 into clean amber bottles and was allowed to stand until all the solvent evaporated at room temperature [17-19].

Results and discussions

Phytochemical screening and Physical Characterization

Phytochemical screening of root bark extract of *T. schimperiana* showed the presence of alkaloids, saponins, Phenols, anthocyanin and tannins [12,20]. In earlier work, methanol extract of leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenol, anthraquinone, glycosides and terpenoids [14,21,22].

Table 1. Result of phytochemical screening

Phytochemical	*n*-Hexan	Ethylacetat	Methanol
Flavonoids	+	+	+
Tannins	+	+	+
Steroids	+	+	+
Carbohydrates	+	+	+
Glycoside	+	+	-
Saponins	-	+	+
Terpenoids	+	+	+
Phenols	-	-	+
Alkaloids	-	-	+
Anthraquinones	-	-	+

+=Present, - =Blow detectable limit

Table 2. Physical characteristic and yield of extracts

Extract	Weight (g)	Colour	Nature	% yield
HE	2.1	Yellow	Oily	0.42
EA	9.9	White yellow	Solid	1.98
ME	34.3	Dark brown	solid	6.86

Table 3. ^{1}H-NMR (400 MHz) data for Stigmasterol Compared with literatures results in $CDCL_3$

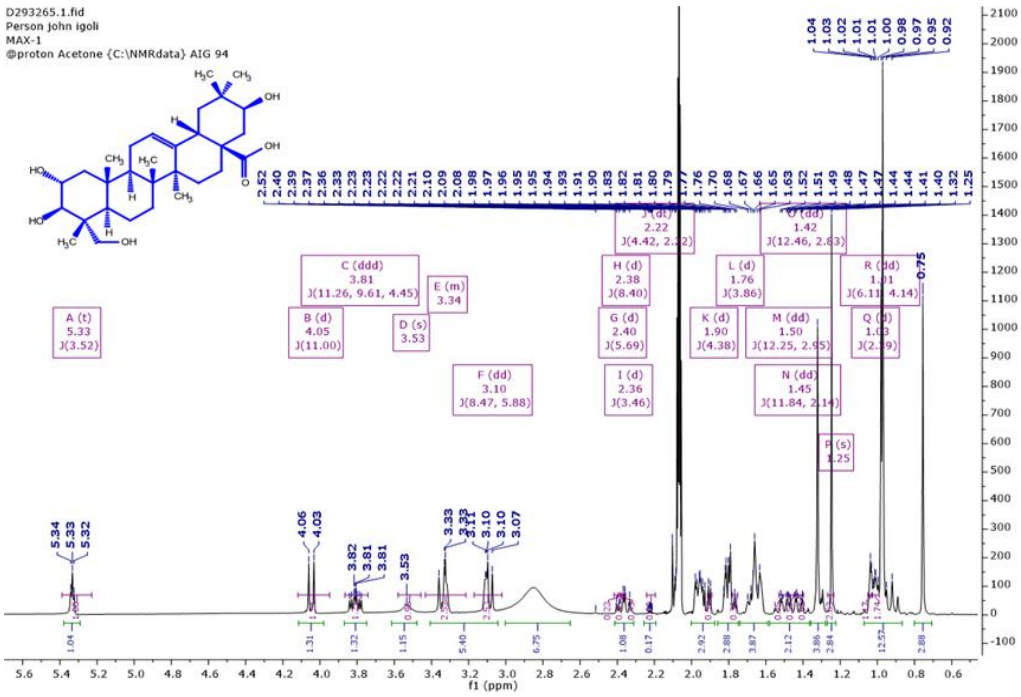
^{1}H NMR (δ) Experimental	^{1}H NMR (δ) Literature[23]	^{1}H NMR (δ) Literature[24]
1.83 (qd, *J*=9.67, 8.42, 4.58H_Z)		
3.52 3.52 (ddd, *J*=15.86, 10.70, 4.57 H_Z)	3.25 (tdd *J*=4.5 H_Z)	3.53 (m, 1H)
5.34 (s, *J*=4.84H_Z)	5.38 (s, 1H)	5.14 (1H, m)
0.68 (s, 3H)	1.29 (d, 3H)	1.07 (3H, s)
1.00 (s, 3H)	0.74 (d, 3H)	1.26 (3H, s)
1.12 (d, 3H)	1.20 (d, 2H)	0.91 (3H, s)
5.14 (dd, *J*=15.15, 8.58H_Z)	5.07 (m, 1H)	4.62 (1H, m)
5.02 (dd, *J*=8.65H_Z)	5.20 (m, 1H)	4.61 (1H, m)
0.82 (s, 3H)	0.84 (d, 3H)	1.01 (3H, s)
0.84 (s, 3H)	0.97 (d, 3H)	1.00 (3H, s)
0.80 (d, 3H)	1.04 (t, 3H)	0.97 (3H, s)

Stigmasterol was isolated as a white-yellow crystal with melting point of 145 °C-147 °C. Its ^{1}H-NMR spectrum indicated resonances for three olefinic methine protons at δ 5.02 (d, *J*=8.65 Hz), 5.14 (dd, *J*=15.15, 8.58 Hz), and 5.34 (d, *J*=4.84 Hz); a carbinol proton at δ 3.52 (ddd, *J*=15.86, 10.70, 4.57); and six methyl protons at δ 0.68 (d, (7.37), 1.00 (s,), 0.83 (d, *J*=1.88 Hz), 0.84 (s), 0.82 (d, *J*=1.88) and 0.80 (s). these signals and assignments are in accordance with the report by [24].

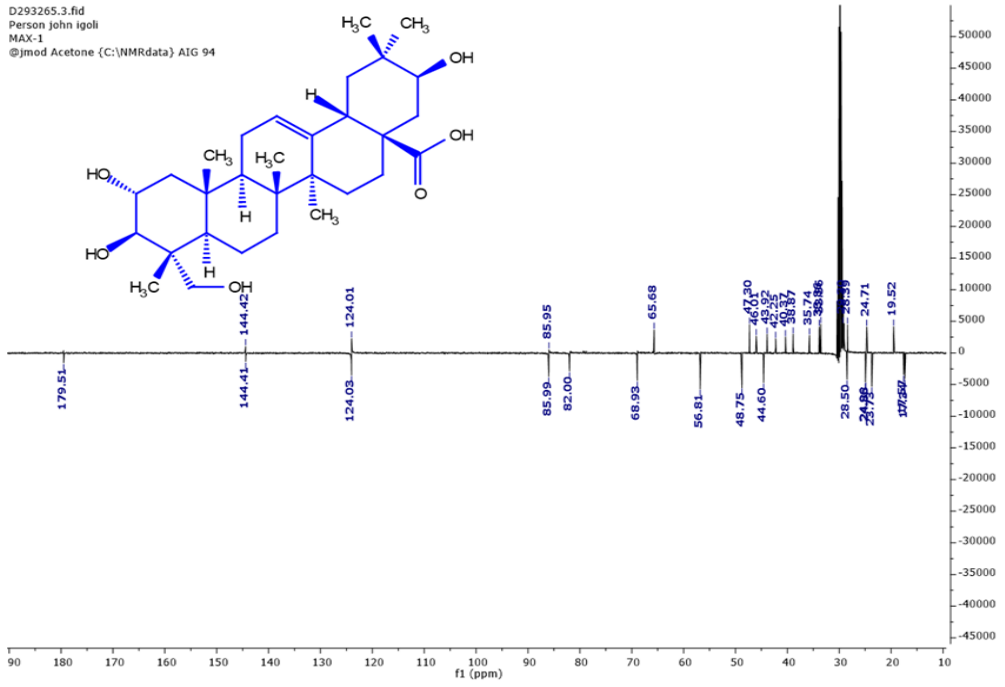
The chemical shift assignments were made on the basis of the information obtained from ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC and comparison of the data with those reported for the corresponding triterpenes [23, 24].

The HMBC spectrum serves to place various functionalities at appropriate places through quaternary carbons. H-18 exhibited long range interactions (3J) with C-12 (δ 123.1), C-16 (δ 27.6) and C-28 (δ 178.6).

The ^{1}H-NMR spectrum indicated resonances for a mixture of two compounds (triglyceride [characteristic glyceryl signals δ 4.12 and 4.29 (sn-1 (stereospecific numbering for glyceryl backbone protons) and sn-3), 5.24 (sn-2); sterol (sterol type methyls 0.67-0.74)] [23-25]. This shows the epimerization of cholesterol to stigmasterol.



(a)



(b)

Figure 2. ^{1}H NMR (a) and ^{13}C NMR (b) of Stigmasterol

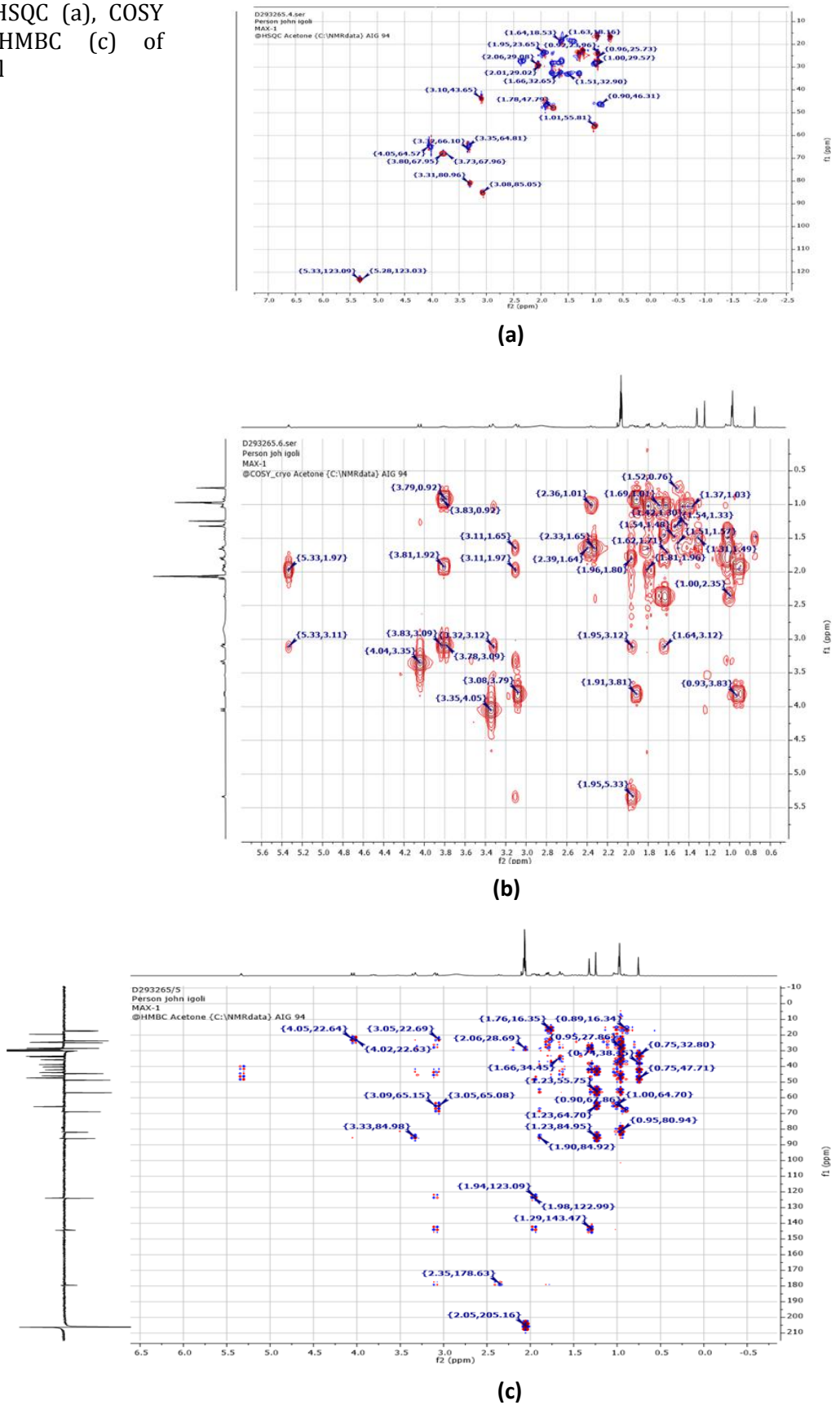


Figure 3. HSQC (a), COSY (b) and HMBC (c) of Stigmasterol

Figure 3. Structure of stigmasterol

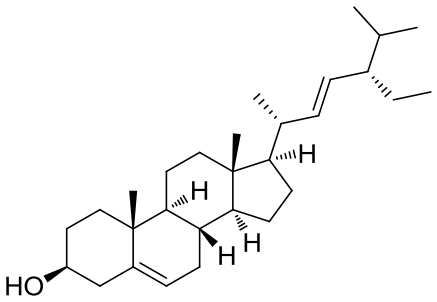
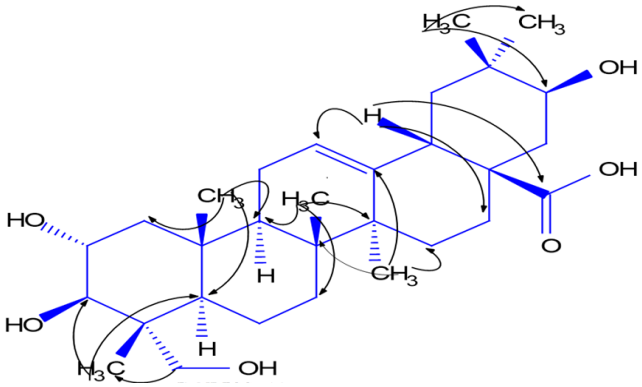


Figure 4. HMBC correlation



The signals/chemical shifts of the white-yellow isolate were recorded successfully based on 1, 2-dimensional NMR spectroscopy. The NMR experimental values of stigmasterol are in agreement with the ones reported by [23,24] as compared in Table 3.

Conclusion

Phytochemical screening of the *n*-hexane, ethylacetate and methanol extracts showed presence of flavonoids, tannins, steroids carbohydrates and terpenoides in all three solvents. Saponins were absent in *n*-hexane. Phenols, alkaloids & anthraquinones absent in *n*-hexane and ethylacetate, while glycosides were absent in methanol. From the ethyl acetate extract of *T. schimperiana*, stigmasterol was successfully isolated for the first time which appeared to be the dominant compound in the roots bark of the plant.

Recommendation

More research should be done on the isolated or purified compound to test for further medicinal- activity. This will help to back-track and compare the activity of both the crude and the pure samples. Also, other solvent extracts should be elucidated for their active principles` activities on microbes

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