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Chemical composition of the leaf essential oil of *Antidesma venosum* E. Mey. ex. Tul. and comparative phytochemical and pharmacognostic analysis of its leaf, stem bark and root

Egharevba H.O.[1*], Dalhatu N.A.[2], Ibrahim J.A.[1]

[1]Deparment of Medicinal Plant Research and Traditional Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria

[2]Department Biological Sciences, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

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Abstract: The leaf of the ethnomedicinally important plant, *Antidesma venosum*, was hydro-distilled to get the essential oil using a Clevenger-type apparatus. The oil was analyzed on Shimadzu QP2010 SE GC-MS to establish the chemical composition. The leaf, stem and bark of root of the plant were extracted and subjected to phytochemical and pharmacognostic analysis to establish their profiles and similarities, using standard methods. The results of the essential oils analysis revealed that the major components included (R)-(+)-Citronellal (28.97%), citronellyl acetate (11.98%), caryophyllene (7.14%), tetradecanal (6.97%), tetracosane (5.96%), caryophyllene oxide (4.65%) and phytol (4.11%). The results of phytochemical screening showed that the stem bark contain saponin, carbohydrate, alkaloid, steroid, tannin and flavonoid while reducing sugar and anthraquinones were absent. The root showed the presence of flavonoids, steroid and saponins, while alkaloids, reducing sugar carbohydrate and anthraquinones were absent. Saponins, carbohydrate, alkaloids, reducing sugars tannins and flavonoid were found in the leaf, while steroid and anthraquinones were absent. The pharmacognostics profile of the leaf revealed that moisture content was 14%, total ash value (8.5%), total solid (86%), water extractive value (4.97%) and alcohol extractive value (19.6%). For the stem bark, the moisture content was 12.1%, ash value (6.6%), total solid (87.9%), water extractive value (4.34%) and alcohol extractive value (8.33%) and for the root moisture content was 13.1%, ash value (8.6%), total solid (86.9%), water extractive value (16%) and alcohol extractive value (45.1%). The phyto-anatomical character (epidermal peeling) carried out on the leaf revealed the presence of parasitic stomata, unicellular trichomes and polygonal cell shape at the lower surface and while stomata and trichomes were absent on the upper surface. The findings of this study establish the bases for its ethnopharmacological applications, and provide information that could be used to determine sample authenticity.

Key words: *Antidesma venosum*, essential oil, Citronellal, Phytochemistry, pharmacognosy

INTRODUCTION

Access and affordability coupled with safety and efficacy perception of the general populace have continued to fuel demand and use of herbal medicinal products all over the world, and most especially, in the developing African communities (Egharevba *et al*., 2015). *Antidesma venosum* E. May ex. Tul. (Euphorbiaceae), is one such medicinal plant that is highly renowned for its demand and use in traditional herbal medicine. The plant which is locally known as *kirni* or *kisni* in Hausa is traditionally used in the treatment of various infectious diseases (Ngishi, 2004). The leaf sap is used for diarrhea and dysentery, the stem bark for dressing wounds while the root decoction is used for syphilis and gonorrheal eruptions (Tor-Anyiin and Yakumbur, 2012). In North central Nigeria, the plant is used for the treatment of typhoid, gastrointestinal disorders and genitourinary tract infection in Anyigba community of Kogi State (Adegoke *et al*., 2013). The methanolic estract of the stem bark had been demonstrated to exhibit mild antimicrobial activities against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* (Tor-Anyiin and Yakumbur, 2012). Adegoke *et al.* (2013) also reported similar antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Salmonella typhi, Streptococcus lactis* and *Shigella sp.* from the methanolic and ethanolic extract of the leaf. *A. venosum* (Figure 1) has also been reported for the treatment of oral candidiasis in Zambezi region of Africa (Chinsembu and Hedimbi, 2010). However, the plant has not been well studied for its chemical constituents. Hence this study intended to comprehensively analyze the different parts of the plant, namely leaf stem and root, which are the mainly used therapeutic recipes and ingredients, with a view toward providing information on the major molecular constituents which could be responsible for some of the observed activities. This is expected to open up a new horizon of research on the plant as a source of new drug.

Figure 1: *Antidesma venosum* E. May ex. Tul.

MATERIALS AND METHODS

Materials

All the solvents and reagents used were of analytical grade and, unless otherwise stated, were sourced from Zayo-Sigma, Abuja, Nigeria. The equipment used for essential oil analysis was GC-MS QP2010 SE Shimadzu, Japan.

***Corresponding Author:**
Egharevba H.O.,
Deparment of Medicinal Plant Research and Traditional Medicine (MPR&TM),
National Institute for Pharmaceutical Research and Development (NIPRD),
Abuja Nigeria.

Plant collection, preparation and extraction

The fresh leaf, stem bark and root of *Antidesma venosum* were collected in August, 2015 from Chaza village, Suleja, Niger State, Nigeria, and authenticated by a Taxonomist in National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The collected parts were sorted and foreign materials were removes by picking and/or rinsing with water. The stem bark and root were chopped into smaller bits. The processes leaf, stem bark and root were separately air-dried for fourteen days and pulverized with aid of a mortar and pestle. The pulverized samples were stored separately in cellophane bags until required.

Hydro-distillation and GC-MS analysis of A. venosum leaf

300g of pulverized leaf material was hydro-distilled over 3 hrs using Clevenger-type apparatus. The oil/water mixture was collected into a glass sample bottle. The mixture was salted with 3 g of sodium chloride salt and then extracted with hexane. The moisture in the hexane extract was removed with 2g of anhydrous Na_2SO_4 and filtered. The hexane filtrate was collected in a glass bottle and subjected to GCMS analysis.

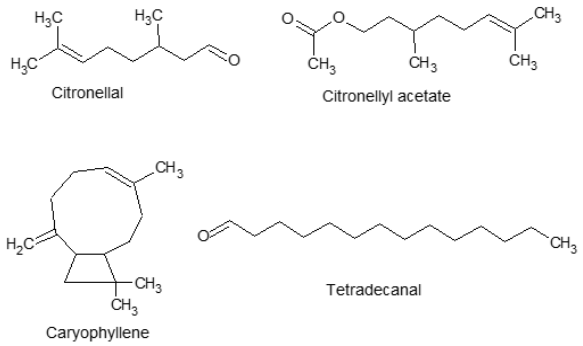
The oil was analyzed on a GC-MS QP2010 SE Shimadzu, Japan at the Shimadzu Training Centre (STC), Lagos, Nigeria in September 2015. The GC was equipped with Optimal 5ms column of length, diameter and film thickness, 30 m, 0.25 mm and 0.25 µm, respectively. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C was raised to 180°C at 10°C/min, held for 2 min and then finally to 280°C at 15°C/min and held for 4 min); injection mode was Split (1.0); injection temperature, 250°C; carrier gas, helium; total flow rate, 6.2mL/min; ion source temperature, 200°C; interface temperature, 250°C; start m/z, 40 and end m/z, 600. The searches and spectral matching of the resolved components were conducted in the National Institute of Standard and Technology (NIST11) database.

Phytochemical and pharmacognostic analyses

The phytochemical and pharmacognostic analysis of the leaf, stem bark and root was carried out to determine the presence of secondary metabolites namely; carbohydrates, anthraquinones, reducing sugars, tannins, alkaloids, saponins and steroids, and proximate pharmacognostic parameters including water and alcohol extractive values, ash values and moisture contents, using standard methods. Microscopic descriptions of the leaf adaxial and abaxial layers were also conducted (Evans, 2002; Sofowora, 2008; Egharevba *et al.*, 2015).

RESULTS AND DISCUSSION

The results of GC-MS analysis of the essential oil of the leaf of *A. venosum* is presented in Figure 2 and Table 1. The GC-MS chromatogram revealed the presence of 40 components constituting 100% of the oil, but only 32 were identified. (R)-(+)-Citronellal was the highest component with a percentage composition of 28.97%, followed by citronellyl acetate (11.98%), caryophyllene (7.14%), tetradecanal (6.97%), tetracosane (5.96%), caryophyllene oxide (4.65%) and phytol (4.11%). There was an unidentified component with a percentage composition of 4.76%. The percentage composition of the other components ranged between 0.03% (citronellol) and 3.24% (eucalyptol or cineole). Citronellal had been reported as insect repellant while its acetate could be used in flavouring (Ruano *et al.*, 2005). Caryophyllene and its oxide are used as food, drug and cosmetic preservative, as well as in flavor and fragrance. Caryophyllene oxides had been reported to possess antimicrobial, inhibitor of photosynthesis and plant growth enhancer (Yang *et al.*, 1999; Sánchez-Muñoz *et al.*, 2012; Sarpietro *et al.*, 2015). Tetradecanal had been reported to exhibit Insect repellant, pesticides and contraceptive activities (Scrivner *et al.*, 1984; Ruano *et al.*, 2005). Phytol had been reported as anti-Schistosomiasis, food additive with non-mutagenic effect, antimicrobial, antinociceptive. It decreases blood cholesterol and increases production of insulin (de Moraes *et al.*, 2014; Nelega 2015; Ghaneian *et al.* 2015). Considering the range of constituents, the essential oil may possess a wide-range of pharmacological and biological activities. This is the first time the compound profile of the essential oil of *A. venosum* leaf will be reported. Though the oil yield was very low, the fact that it was rich in citronellal and its derivative could encourage genetic modification for exploitation as a biological source of the compounds.



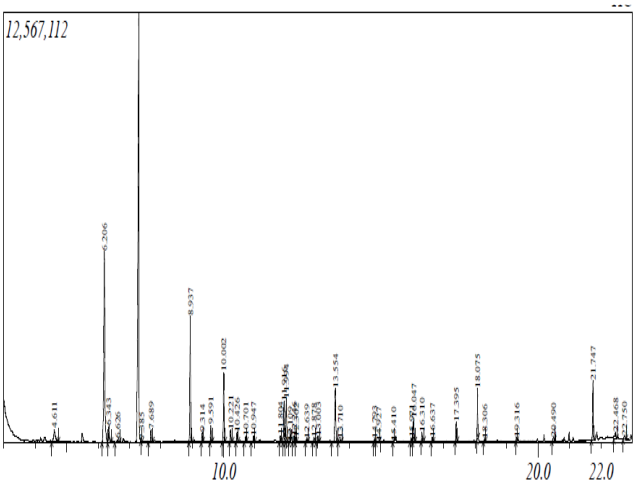


Figure 2: GC Chromatogram of the essential oil of *A. venosum* leaf

Table 1: Results of GC-MS analysis of the essential oil of *A. venosum* leaf

S/N	Retention Time (min)	% content (Area of peaks)	MW (Da)	Names of Compounds
1	4.611	3.24	154	Eucalyptol
2	6.206	28.97	154	(R)-(+)-Citronellal
3	6.343	2.16	154	Isopulegol
4	6.626	0.32	154	NI*
5	7.385	0.03	156	Citronellol
6	7.689	1.29	184	Methyl citronellate
7	8.937	11.98	198	Citronellyl acetate
8	9.314	0.87	196	Neryl acetate
9	9.591	1.68	204	[1S-(1α,2β,4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane
10	10.002	7.14	204	Caryophyllene
11	10.221	1.13	194	*cis*-Geranylacetone
12	10.426	0.94	204	Humulene
13	10.701	0.50	192	*trans*-β-Ionone
14	10.947	0.63	204	α-Farnesene
15	11.804	0.56	192	(Z)-2,6,10-trimethyl-1,5,9-Undecatriene
16	11.916	4.76		NI*
17	11.994	4.65	220	Caryophyllene oxide
18	12.109	0.08		NI*
19	12.246	0.57	222	Farnesol
20	12.302	0.41		NI*
21	12.639	0.40		NI*
22	12.878	0.58	204	β-Eudesmene
23	13.003	0.73	200	n-Tridecan-1-ol
24	13.554	6.97	212	Tetradecanal
25	13.710	0.24	204	(E)-β-Farnesene
26	14.793	0.24	210	(Z)-7-Tetradecenal
27	14.927	0.11	252	14-Heptadecenal
28	15.410	0.07	268	Hexahydrofarnesyl acetone
29	15.971	0.41	144	*cis*-1,2-Cyclohexanedimethanol
30	16.047	2.60	238	(Z)-7-Hexadecenal,
31	16.310	0.85	262	Farnesyl acetone
32	16.637	0.38	156	Neo-Menthol
33	17.395	1.63	290	3,7,11,15-tetramethyl-(E,E)-1,6,10,14-Hexadecatetraen-3-ol
34	18.075	4.11	296	Phytol
35	18.306	0.25		NI*
36	19.316	0.29		NI*
37	20.490	0.56	408	Heneicosane
38	21.747	5.96	338	Tetracosane
39	22.468	1.11	310	Docosane
40	22.750	0.57		NI*

NI* = Not identified

The results of phytochemical screening revealed the presence of carbohydrates in the leaf and stem, reducing sugars in the leaf only, tannins, saponins and flavonoids in the leaf, stem bark and root, alkaloid in the leaf and stem bark, and steroids in the stem bark and root (Table 2). Thus the stem bark is more similar to the leaf in the profile of secondary metabolites than the root as expected. The pharmacognostic profile (Table 3) revealed that the moisture contents among the three parts were within the range of 8-14 prescribed for vegetable drug (Egharevba *et al.*, 2015). Alcohol was generally a better solvent for extraction than water, and the ash values were higher in the leaf (8.5%) and root (8.6%) than in the stem (6.6%). The microscopic description of the foliar characteristics is shown in Table 4 and Figure 3 & 4. The upper epidermal layer (adaxial) did not contain stomata and trichomes while parasitic stomata cells and unicellular trichomes were present in the lower epidermal layer (abaxial). The adaxial epidermal cells were circular and polygonal while the abaxial epidermal cells were tetrahedral and polygonal in shape. These findings will be useful in monograph development and samples identification and authentication.

Table 2: Phytochemical screening on *A. venosum* leaf, stem bark and root

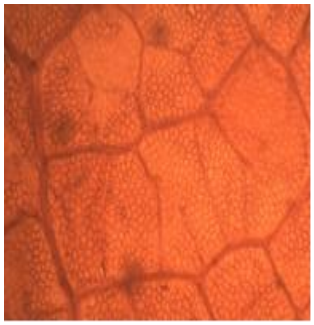
Metabolites	Leaf	Stem bark	Root
Carbohydrates	+	+	-
Anthraquinones	-	-	-
Reducing sugars	+	-	-
Tannins	+	+	+
Alkaloids	+	+	-
Saponins	+	+	+
Steroids	-	+	+
Flavonoids	+	+	+

Table 3: Results of pharmacognostic analyses of *A. venosum* leaf, stem bark and root.

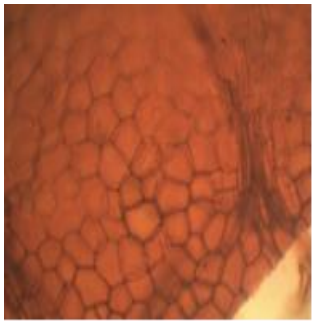
Parameters	Leaves	Stem	Root
Moisture Content (%)	14	12.1	13.1
Total Solid (%)	86	87.9	86.9
Water Extractive Value (%)	4.97	4.34	16.207
Alcohol Extractive Value (%)	19.6	8.33	45.1
Total Ash Value (%)	8.5	6.6	8.6

Table 4: Result of microscopic study *A. venosum* leaf showing the foliar epidermal characters

Characters	Adaxial (Upper Surface)	Abaxial (Lower Surface)
Cell shape	Circular and polygonal epidermal cell shape.	Tetrahedral and polygonal epidermal cell shape
Trichomes	Absent	Present
Trichome type	Absent	Unicellular trichome
Stomata	Absent	Present
Stomata type	Absent	Parasitic stomata

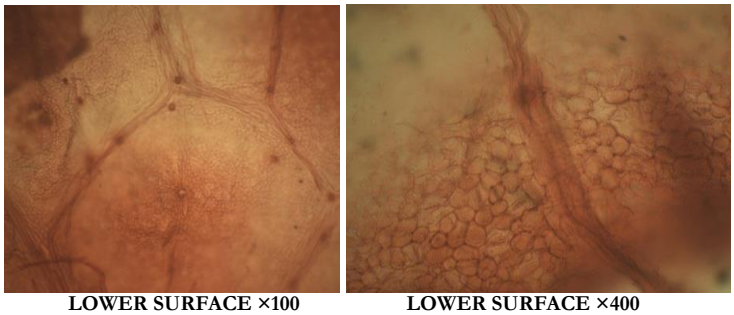


UPPER SURFACE × 100



UPPER SURFACE × 400

Figure 3: microscopy of *A. venosum* leaf the upper layer (Adaxial) showing polygonal cells with no stomata and no trichomes



LOWER SURFACE ×100 LOWER SURFACE ×400

Figure 4: Microscopy of *A. venosum* leaf the lower layer (Abaxial) showing cell circular, polygonal cells, stomata and trichomes

CONCLUSION

The essential oil of the leaf of *A. venosum* is rich in citronellal, citronellyl acetate, caryophyllene, tetradecanal, tetracosane, caryophyllene oxide and phytol. This could serve as profile for authentication of the leaf sample. The leaf, stem and root also contain important phytochemical that may be responsible for some of the biological activities associated with the plant in ethnomedicine. The information could also expand the horizon of it application in herbal medicine as well as open up new research in the development of the plant as a herbal drug or natural source of biomolecules such as citronellal and its derivatives. Phamacognostic and microscopic description of the plant could help in sample identification and authentication.

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