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Full Length Research Paper

GC-MS analysis of leaf, stem-bark and root extracts of *Alstonia boonei*

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Alstonia boonei **De Wild is a medicinal plant commonly found in West African and is popularly known as God's tree. The plant parts have been traditionally used as a painkiller, antimicrobial, antimalarial and antidiabetic which have also been proved scientifically. Previous studies revealed little information on phytochemcial components of *A. boonei.* The present work aims at investigating and comparing the chemical components of the leaf, the stem-bark and the root of the plant in order to provide sufficient baseline information for isolation work on medicinal components of the plant. Leaf, stem-bark and root extracts of *A. boonei* were prepared by maceration using 1:1 EtOAc/MeOH. The crude extract was successively macerated with hexane, dichloromethane (DCM) and methanol. Thin layer chromatography (TLC) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) (TLC-DPPH) analysis was used to screening out DCM fraction for further analysis. Gas chromatography and mass spectroscopy studies were performed to profile phytochemical constituent of the plant. The GC-MS analysis of DCM extract of the leaf** revealed **ten chemical components with Eugenol as major component (54.58%); DCM extract of the stem-bark showed forty one components with alpha-amyrin (32.25%) while DCM extract of the root revealed twenty components with 1,2-benzenedicarboxylic acid (49.2%) as major component. This study shows that the *A. boonei* extracts of the leaf, stem-bark and root consist of different types of compounds with few components common to two of the parts. Quantitatively, common phytochemicals decrease from leave to root. The most prominent compounds identified by GC/MS were Eugenol, benzenedicarboxylic acid and alpha-amyrin.**

Key words: Phytochemicals, *A. boonei*, gas chromatography/mass spectroscopic studies, Eugenol

INTRODUCTION

Alstonia boonei De Wild belongs to the family Apocynaceae. It is a herbal medicinal plant of West African origin, popularly known as God's tree, cheese wood and known locally among Yoruba in Nigeria as Ahun. All the parts of the plant are very useful but the thick bark cut from the matured tree is the part that is most commonly used for therapeutic purposes. Therapeutically, beta-amyrin and alpha-amyrin acetate isolated from the stem bark of *A. boonei* have been found to possess anti-inflammatory properties (Okoye et al.,

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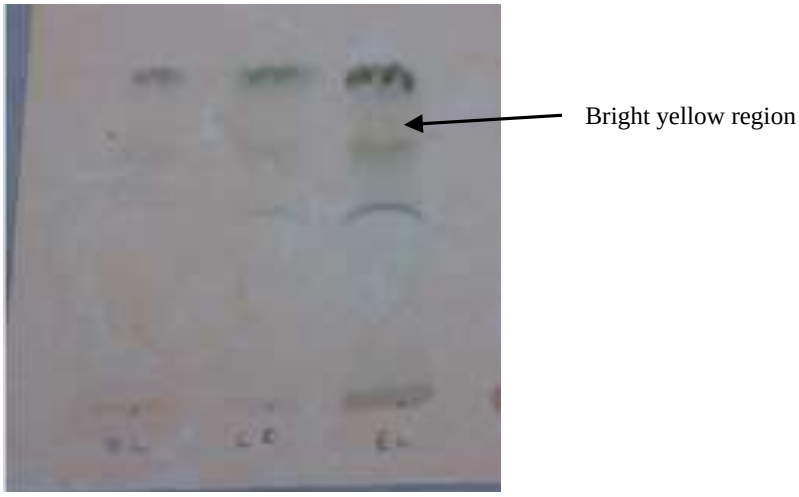

Figure 1. SL = Hex LC = MeOH, EL = DCM

2014) Antimicrobial activities of various fractions of stem bark of *A. boonei* were also tested against some microbes (Bello et al., 2009; Amole and Ilori, 2010; Misra et al., 2011). A wide array of chemical compounds have been isolated from *A. boonei*. These include alkaloids, tannins, iridoids, and triterpenoids which have been demonstrated to possess some pharmacological activities (Akinmoladun et al., 2007). The alkaloids isolated from the plant include echitamine and other alkaloids, and the triterpenes b-amyrin, lupenol, and ursolic acid have all been isolated from leaves and stem bark (Adotey et al., 2012). Echitamine possess a battery of pharmacological and autonomic activities (Ojewole, 1983, 1984) including anticancer activities (Adotey et al., 2012; Ashok et al., 2015). Moronkola and Kunle (2012) reported essential oil compositions of the leaf, stem bark, and root of *A. boonei*. (Z)-9-Octadecenoic acid was found to be the most abundant volatile oil in the leaf and stem bark while methyl (7 E)- 7-octadecenoate was the most abundant in the root. The aim of this study was to investigate the distribution trend of *A. boonei* chemical composition

MATERIALS AND METHODS

Sample preparation

A. boonei leaf, stem-bark and root were collected at a farmland in Akinmarin village, Oyo and were identified at the Department of Botany, University of Ibadan, Ibadan. The air-dried plant materials: (204.7 g) leaves, (282.5 g) stem-bark, and (102.9 g) root of, *A. boonei*, were exhaustively extracted separately with 1:1 EtOAc:MeOH for 3 days. The extracts were filtered separately, concentrated on rotary evaporator to about 50 ml and evaporated to dryness under vacuum. Each extract was successively macerated with hexane, dichloromethane and methanol. Obtained fractions were evaporated to dryness and stored in a refrigerator at +4°C until use.

Antioxidant screening

The method of Mensor et al. (2001) with Kotze and Eloff (2002) methods were adopted to screen fractions. Fractions obtained from leaves were developed on aluminium-backed thin layer chromatography (TLC) using solvent ratio: chloroform/ethyl acetate/formic acid (5:4:1) (intermediate polarity/acidic). Chromatogram was sprayed with 0.2%, 2,2, diphenyl-2-picryl-hydrazyl to detect antioxidant properties of various fractions.

Gas chromatography (GC)–mass spectrometer (MS) analysis

GC-MS: Hewlett-Packard 5890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer with source at 270°C at 70 eV. Injector was set at 270°C with splitting ratio 1:30. The analysis was performed on the aforementioned programme on equivalent column HP-5 (25 m × 0.22 mm and 0.25 µm). A mass spectral survey was performed using the NIST mass spectral search program.

RESULTS AND DISCUSSION

Qualitative analysis of antioxidant activity was used to screen out less potent fraction. The dichloromethane fraction displayed highest antioxidant activity after spraying chromatogram with DPPH because of the intensity of yellow colour produce. Dichloromethane (DCM) was then selected for phyto-constituent analysis. Thin layer chromatogram of hexane, dichloromethane and methanol fractions obtained from the leaves is shown in Figure 1. GC-MS analyses of DCM fraction of the leaf, the stem-bark and the root column of *A. boonei* are presented in Table 1. Constituents were listed in order of elution from HP-5 capillary column (Table 1). Gas chromatography-mass spectrometry analysis of the leaf extract resulted in the identification of 10 compounds, 41 compounds in stem-bark extract and 20 compounds in root extract.

Table 1. Chemical composition of leaf, stem-bark and root of *Alstonia boonei.*

S/N	Constituent	% composition		
		Leaf	Stem-bark	Root
1	Eugenol	54.58(9.730)	0.14(9.722)	-
2	4-Tetradecene	-	-	1.39(10.185)
3	Caryophyllene	6.99(10.631)	-	-
4	Phenol	2.01(12.148)	-	0.56(11.970)
5	1H-2-Benzopyran-1-one	-	0.54(12.434)	-
6	Cyclododecane	-	0.28(12.943)	-
7	Dichloroacetic acid	-	-	4.83(12.943)
8	Hexadecane	-	0.16(13.033)	1.32(13.029)
9	2H-Pyran-2-one	-	1.54(13.161)	-
10	2-Ethyl-3-methoxypyrazine	-	0.34(14.334)	-
11	1-Octadecene	-	0.37(15.324)	6.59(15.323)
12	Octadecane	-	0.15(15.398)	1.35(15.392)
13	Cyclononasiloxane	-	0.20(15.713)	-
14	Bicyclo(3.1.1)heptane	2.61(15.833)	-	-
15	1,2-Benzenedicarboxylic acid	-	-	49.20(16.210)
16	Phthalic acid	-	0.15(16.216)	-
17	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	-	-	1.13(16.777)
18	Hexadecanoic acid	2.13(16.765)	0.67(16.777)	-
19	n-Hexadecanoic acid	5.40(17.183)	1.40(17.303)	-
20	Dibutyl phthalate	-	-	2.61(17.200)
21	6H-Furo[2',3':4,5] oxazolo [3,2-a]pyrimidin-6-one	-	0.24(17.338)	-
22	Heptafluorobutyric acid	-	0.25(17.458)	-
23	n-Nonadecanol-1	-	0.28(18.391)	5.90(17.452)
24	Eicosane	-	0.16(20.365)	1.34(17.509)
25	Ethanone	-	-	1.30(17.767)
26	1-Heneicosanol	2.88(17.950)	-	-
27	Behenic alcohol	-	-	3.21(18.373)
28	9,12-Octadecadienoic acid	-	0.34(18.488)	-
29	11-Octadecenoic acid	-	0.26(18.545)	-
30	Phytol	1.63(18.665)	-	-
31	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	-	0.33(18.820)	-
32	Oleic Acid	-	0.46(18.986)	-
33	1-Octadecene	-	0.19(19.192)	-
34	1-Docosene	13.28(22.585)	-	3.65(19.392)
35	2-Eicosanol	-	0.14(19.398)	-
36	Docosane	-	-	0.66(19.438)
37	6-Acetamido-2-methylbenzothiazole	-	0.78(20.090)	-
38	Cyclotetracosane	-	-	2.56(21.337)
39	Hexasiloxane	-	0.40(21.686)	-
40	2(3H)-Furanone	-	-	0.82(22.430)
41	1-Heptadecene	-	-	0.92(22.659)
42	Tetratetracontane	-	0.95(22.693)	-
43	Oxirane	-	0.27(23.186)	-
44	Olean-12-ene	-	0.63(24.948)	-
45	1S,6R,9S)-5,5,9,10-Tetramethyltricyclo [7.3.0.0(1,6)] dodec-10(11)-en	-	1.29(25.245)	-
46	12-Oleanen-3-yl acetate	-	0.66(25.366)	-
47	2H-Cyclopropa[a]naphthalen-2-one	-	1.10(25.474)	-
48	Heptadecane	-	-	1.53(26.304)
49	Stigmasterol	-	0.22(29.182)	-
50	2,6,10,14,18,22-Tetracosahexaene	8.50(29.829)	-	-

Table 1. Cont'd

51	5(1H)-Azulenone	-	1.53(30.401)	-
52	Urs-12-en-24-oic acid	-	0.50(30.447)	-
53	Urs-12-en-3-ol	-	7.76(30.922)	-
54	2-Isopropenyl-4a,8-dimethyl-1,2,3, 4,4a,5,6,8a-octahydronaphthalene	-	2.44(32.009)	-
55	Nonacosane	-	0.68(32.524)	-
56	Azuleno[6,5-b]furan-2,5-dione	-	-	4.15(33.439)
57	Bicyclo[2.2.1]heptane	-	3.89(33.582)	-
58	alpha.-Amyrin	-	32.25(35.276)	-
59	Pyrrolo[2,3-b]indole	-	4.05(36.049)	-
60	2(1H)Naphthalenone	-	10.32(36.094)	-
61	9,19-Cycloergost-24(28)-en-3-ol	-	9.13(37.771)	-

Note: Values in bracket are retention time

Table 2. Comparative analysis of chemical profiles of the whole *Alstonia boonei* plant.

S/N	Constituent	% composition		
		Leaf	Stem-bark	Root
1	Eugenol	54.58(9.730)	0.14(9.722)	-
2	Phenol	2.01(12.148)	-	0.56(11.970)
3	Hexadecane	-	0.16(13.033)	1.32(13.029)
4	1-Octadecene	-	0.37(15.324)	6.59(15.323)
5	Octadecane	-	0.15(15.398)	1.35(15.392)
6	Hexadecanoic acid	2.13(16.765)	0.67(16.777)	-
7	n-Hexadecanoic acid	5.40(17.183)	1.40(17.303)	-
8	n-Nonadecanol-1	-	0.28(18.391)	5.90(17.452)
9	Eicosane	-	0.16(20.365)	1.34(17.509)
10	1-Docosene	13.28(22.585)	-	3.65(19.392)

Note: Values in bracket are retention time

Chemical compounds that were common to the three parts of the plant were presented in Table 2. A comparative analysis of chemical profiles of the leaf and stem-bark showed that hexadecanoic acid and n-hexadecanoic acid were present in the two parts but in higher proportion in the leaf while phenol and 1-Docosene were present in the leaf and the root. Hexadecane, 1-Octadecene, Octadecane, n-Nonadecanol-1 and Eicosane were present in both stem-bark and root. There is decrease in quantity of common phytochemicals from leave to root and root to stem. The results revealed that Eugenol (54.58), 1-Docosene (13.28), 2,6,10,14,18,22-Tetracosahexaene (8.50), Caryophyllene (6.99) and n-Hexadecanoic acid (5.40) were found as the five major components in the leaf extract, the minor compound was Phytol. The major constituents of stem-bark extract were alpha-amyrin (32.25), 2(1H) Naphthalenone (10.32), 9,19-Cycloergost-24(28)-en-3-ol (9.13) and Urs-12-en-3-ol (7.76) while the major component found in root were 1,2-Benzenedicarboxylic acid (49.20), 1-Octadecene(6.59) And n-Nonadecanol-1(5.90).

Jaganathan and Supriyanto (2012) reported that eugenol possess anticancer activity against various types of cancers. Bioactivity experiments revealed positive anti-cancer activity of 1, 2-Benzenedicarboxylic acid on PC3, MCF, HCT-116, A549, and MIAPACA cell lines (Save et al., 2015). Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of *A. boonei* display profound anti-inflammatory activity (Nkeoma et al., 2014).

Conclusion

The present study represents the comprehensive analysis of phyto-constituents of *A. boonei* leaf, stem-bark, and root DCM extracts. Percentage composition of common phytochemicals decreases from leaf to root. Previous study revealed that the three main components of *A. boonei* identified by GC-MS exhibit anticancer activity. Therefore, the results of this study will form the basis for selection of plant part for further investigation in

the potential drug discovery.

CONFLICT OF INTEREST

The author has not declared any conflict of interest.

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