

Full Length Research Paper

Gas chromatography-mass spectrometric analysis of methanolic leaf extract of *Blighia sapida*

Theresa I. Edewor* and Nimotalai O. Kazeem

Department of Pure and Applied Chemistry, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Blighia sapida is a medicinal plant whose fruits and leaves possess therapeutic properties. This research is aimed at identifying the phytochemical constituents present in the leaves. The plant leaves were extracted with n-hexane and methanol. Phytochemical screening was carried out on both extracts while only the methanolic extract was subjected to gas chromatography-mass spectrometric (GC-MS) analysis. The phytochemical screening revealed the presence of saponins and steroids in the n-hexane extract while tannins, steroids and flavonoids were present in the methanolic extract. Twenty one compounds were separated and identified using GC-MS. The predominant compounds were ethanol, 2-butoxy-(23.283%), n-hexadecanoic acid (12.214%) and phytol (9.803%). Some of the identified compounds possess biological properties. The presence of these compounds in the leaves of *B. sapida* confirms its ethnomedical uses.

Key words: *Blighia sapida*, medicinal, leaves, ethnobotanical, phytochemicals, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

Health is as important to man as his food. The maintenance of man's health has gone beyond the use of only synthetic drugs to the use of plant materials which could serve as both food and drug. Synthetic drugs either have side effects that affect the consumer negatively or are too expensive. A safe alternative is to resort to natural products such as plant materials whose potency in alleviation of ailments have been proved by numerous researches. There are several medicinal plants around the world and 15% of these have been screened for their therapeutic values (Hatta and Shafei, 2013). Herbal drugs can either cure or act as supplement. Some

people consume herbal drugs so as to aid the body system in its function (Praveena and Suriyavathana, 2013). Medicinal plants can serve as raw material base for the production of semi-synthetic drugs. The growing awareness of the medicinal importance of plants has led scientist to find correlations between the phytochemicals present in a plant and its pharmacological activity (Hussain and Kumaresan, 2013; Ashurst, 1871).

Blighia sapida also known as Ackee is a tree that grows across West African countries. It grows to a height of about 10 m. The leaves are 15 to 30 cm in length and 5 to 8 cm wide. The fruit is pear shaped and green but turns red

*Corresponding author. E-mail: tiedewor@lautech.edu.ng.

when ripe. It contains three shiny seeds. *B. sapida* is a member of the Sapindaceae family of plants. Much research has been carried out on the fruit which is eaten as food in some African countries, Jamaica and other South American countries (Morton, 1987; Atolani, 2001). The unripe fruit is known to contain some poisonous substances known as hypoglycins (Atolani et al., 2009; Ubulom et al., 2012). The aqueous extract of the seed is used for the treatment of headache, cold and expulsion of parasites while the leaf extract is used to treat diarrhea (Gbolade, 2009), diabetics (Ekué et al., 2010), dysentery, cold and pains (Mitchell and Ahmad, 2001). Other uses include ascaricidal and insecticidal (Kean E. A and Hare, 1980); the juice from the leaves is used as eye drops in ophthalmia and conjunctivitis (Gbile and Soladoye, 2002). The aim of this research work is to determine the phytochemicals that are present in the leaves of *B. sapida* and thereby ascertain its ethnobotanical use.

EXPERIMENTAL

Sample collection and preparation

The plant leaves were collected from a medicinal farm in Egbe, Kogi State, Nigeria. The plant was shown to Prof. S. O. Owa of Landmark University, Omu Aran, Kwara State, Nigeria, who got the local name and the picture of the plant and identified the plant from the book by Z. O. Gbile and M. O. Soladoye (Harborne, 1993). The leaves were air dried in the laboratory for two weeks and pulverized using a sterilized food blender. All solvents used were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were distilled before use.

Extraction

The plant leaves were extracted with n-hexane and later with methanol using a Soxhlet extractor. The extract was concentrated by distillation and later evaporated to dryness.

Phytochemical analysis

Harborne (1993)¹⁵ method which is for determination of the presence of phytochemicals in a crude extract was used to analyze the crude extracts for the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, steroids and glycosides.

Gas chromatographic-mass spectrometric analysis

The crude extract was subjected to GC-MS. Model 7890A; Agilent Technologies interfaced with mass selector detector (MSD) model 5975C was used. 5% Diphenyl and 95% dimethyl siloxane were used to pack the column. The sample was dissolved in methanol and 1 µl of the sample was taken and separately used for the analysis. The samples were injected in the split mode with ratio 50: 1. Helium was used as the carrier gas which had a constant flow rate of 1.5 ml/min. The injection temperature was 300°C while the total running time was 49 min. The ionization voltage was kept at 70 eV. The oven temperature was kept at 80°C, held for 4 min and ramped to 270°C at the rate of 3.5°C/min holding for 6 min.

RESULTS AND DISCUSSION

The identification of phytochemicals present in plant materials which are used either for food, cosmetics and medicine is very important. This can serve as a source of information in the discovery of therapeutic drugs and the disclosure of new sources of economic phytochemicals which can be modified through synthesis to produce complex chemical substances that will be beneficial to human health. *B. sapida* is a medicinal plant and it is important to have knowledge of the phytochemicals that are present in the plant. Phytochemical analysis of the leaf extracts identified saponins and steroids in the n-hexane extract while tannins, steroids and flavonoids were present in the methanol extract shown in Table 1. These phytochemicals have been reported to possess antidiarrhoeal, antihemorrhagic (Dhivya and Manimegalai, 2013; Priya et al., 2011), anti-inflammatory (Salini and Shankar, 2014), antioxidant, antimicrobial and health promoting properties (Table 1).

Phytochemical screening of the crude extracts of *B. sapida*

The total ion chromatogram of the methanolic leaf extract of *B. sapida* showing the separated compounds with their peak areas and retention times is shown in Figure 1.

The GC-MS analysis of the methanolic extract revealed the presence of twenty one compounds shown in Table 2. The first compound to emerge from the GC was o-xylene with retention time of 5.291 min, while the last to emerge was squalene with retention time of 44.780 min. The predominant phytochemicals are ethanol, 2-butoxy- (23.283%), n-hexadecanoic acid (12.214%) and phytol (9.803%). These compounds were identified based on their peak area, retention time, molecular formula, molecular structure, molecular mass, and calculated fragments. The interpretation of the mass spectra of the unknown compounds was carried out by comparing the mass spectrum of the unknown compound with a matching spectrum obtained from NIST 2011 database incorporated into the computer system of the GC-MS equipment. Identified bioactive phytoconstituents such as phytol and n-hexadecanoic acid have also been identified in the leaves of *Woodfordia fruticosa* (Grover and Patni, 2013), *Kedrostis foetidissima* (Kalaisezhiyen and Sasikumar, 2012) and *Aloe vera* (Arunkumar and Muthuselvam, 2009). The presence of phytol in the plant leaves confirms its use as an antimicrobial and anti-inflammatory agent. Squalene has been reported to possess antioxidant, antitumor and anticancer properties (Salini and Shankar, 2014). Some of the identified compounds have been reported to exhibit biological properties shown in Table 3.

The presence of these compounds in the plant leaves

Table 1. Phytochemical screening of the crude extracts of *Blighia sapida*.

Extract	Alkaloids	Saponins	Steroids	Terpenoids	Tannins	Flavonoids	Glycosides
n-Hexane	+	-	+	-	-	-	-
Methanol	-	-	+	+	+	+	-

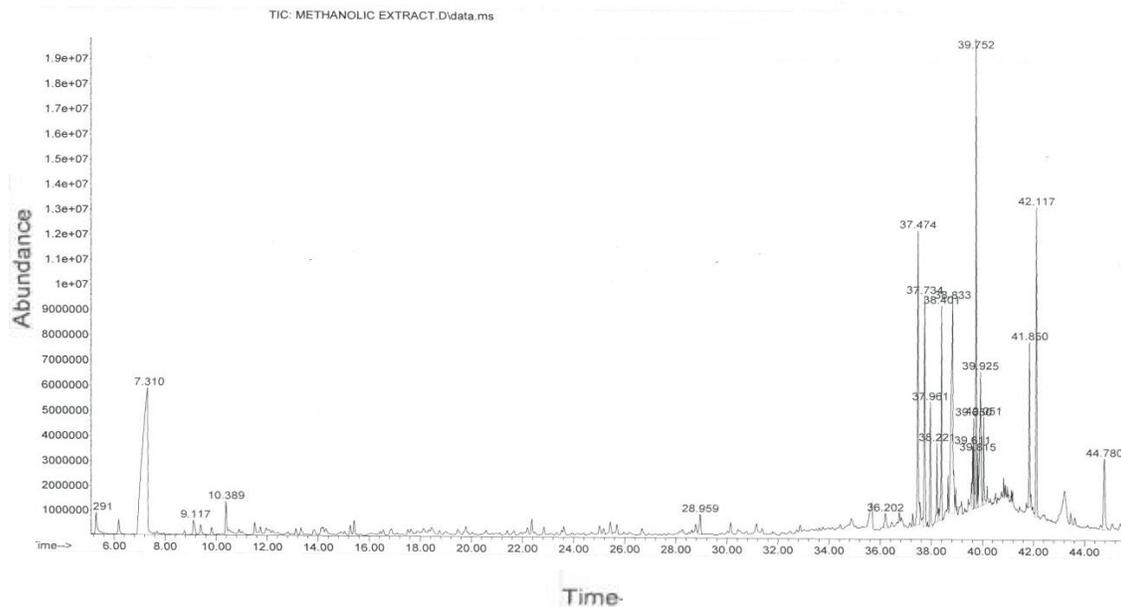


Figure 1. Total ion chromatogram of the methanolic leaf extract of *Blighia sapida*.

Table 2. GC-MS analytical report on the methanolic crude extract of *Blighia sapida*.

S/N	Retention time	Name of compound	Molecular weight	Molecular formula
1	5.291	o-Xylene	106	C ₈ H ₁₀
2	7.310	Ethanol, 2-butoxy-	118	C ₆ H ₁₄ O ₂
3	9.117	Benzene, 1-ethyl-3-methyl-	120	C ₉ H ₁₂
4	10.389	Benzene, 1, 2, 3-trimethyl-	120	C ₉ H ₁₂
5	28.959	Naphthalene, 1, 2, 4a, 5, 8, 8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1a, 4aa, 8aa)]-	204	C ₁₃ H ₂₄
6	36.202	Tetradecanoic acid	228	C ₁₄ H ₂₈ O ₂
7	37.474	10-Heptadecen-8-ynoic acid, methyl ester	278	C ₁₈ H ₃₀ O ₂
8	37.734	1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl)	278	C ₁₈ H ₂₆ O ₄
9	37.961	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	296	C ₂₀ H ₄₀ O
10	38.221	Phthalic acid, butyl-2-ethylbutyl ester	306	C ₁₈ H ₂₆ O ₄
11	38.401	Hexadecanoic acid, methyl ester	270	C ₁₇ H ₃₄ O ₂
12	38.833	n-hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂
13	39.611	8, 11-octadecadienoic acid, methyl ester	294	C ₁₉ H ₃₄ O ₂
14	39.650	9, 12-octadecadienoyl chloride, (Z, Z)-	298	C ₁₈ H ₃₁ ClO
15	39.752	Phytol	296	C ₁₂₀ H ₄₀ O
16	39.815	Heptadecanoic acid, 16-methyl- methyl ester	298	C ₁₉ H ₃₈ O ₂
17	39.925	17-octadecynoic acid	280	C ₁₈ H ₃₂ O ₂

Table 3. Biological properties of *Blighia sapida* methanolic leaf extract.

S/N	Name of compound	Type	Properties
1	Squalene	Triterpene	Antibacterial, antioxidant, antitumor, immunostimulant, lipooxygenase inhibitor Pesticide
2	Phytol	Diterpene	Antimicrobial, anticancer, diuretic, anti-inflammatory
3	9, 12-Octadecadienoyl chloride, (Z, Z)-	Linoleic acid	Antiinflammatory, nematocide, insectifuge, hypocholesterolemic, cancer preventive, hepatoprotective, antihistaminic, antiacne, anti-arthritic, antieczemic, 5 α -reductase inhibitor, antiandrogenic
4	n-Hexadecanoic acid	Fatty acid	Antioxidant, nematocide, insectifuge, hypocholesterolemic, 5 α -reductase inhibitor
5	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic.
6	3, 7, 11, 15 - Teramethyl- 2-hexadecen-1-ol	Terpene alcohol	Antimicrobial

Source: Dukes. Phytochemical and Ethnobotanical Databases. Phytochemical and Ethnobotanical Databases. www.ars-gov/cgi-bin/duke/. 2013.

confirm its use against dysentery, diarrhea, insects and nematodes.

Conclusion

The methanolic leaf extract of *B. sapida* contains terpenes, fatty acids, fatty acid esters, other esters, terpene alcohol and aromatic compounds. The biological properties of the plant leaves are in agreement with its tradomedical use. Research is on-going to isolate and determine biological and pharmacological parameters of the phytoconstituents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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