Isolation and Characterization of Bioactive Constituents of *Boscia salicifolia* Olive (Capparaceae)

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ABSTRACT

Extraction of the crude extract of B. salicifolia was carried out using methanol as solvent with the aid of Soxhlet apparatus. The extract was concentrated in-vacuo at 40^oC using rotary evaporator. The crude methanol extract was subjected to thin layer chromatography and vacuum liquid chromatography (VLC). The isolated compound BSME was characterized by IR and GC-MS. The IR spectral studies indicated the presence of hydroxyl, carbonyl, alkane and aromatic functional groups. GC-MS revealed nine prominent peaks with retention times of 5.133, 11.62, 14.692, 15.725, 16.475, 17.433, 18.183, 18.317 and 20.733 minutes indicating the presence of 1,2,3-propanetriol, 3,5-diterbutylphenol, 2-(2-hydroxy-2-phenylethoxy)phenol, methyl-12-methyltetradecanoate, n-Hexadecanioc acid, 13-octadecanoate, 9- Octadecanioc acid, n-Octadecanoic acid and 9- octadecenal respectively.

INTRODUCTION

The use of plants for medicinal purposes dates back to thousands of years ago and one of the great virtues of medicinal plants is precisely their ability to regulate life processes and prevent diseases[1]. Herbal medicine sometimes referred to as Herbalism or phyto-therapy is a folk medicine practice based on the use of plants and plant extracts. The plants are able to synthesize a wide variety of chemical compounds of conceivable class and some of the compounds exhibit marked pharmacological activities. Strivastava and Viet, (1996)[2] reported that hundreds of plant species have been recognized as having medicinal values and some of them are from the floras of developing countries. He stated also that medicinal properties may be found in the plant root, stem, bark, leaf, fruit or seed. Prehistoric people primarily depended on plants for their survival.

Boscia salicifolia is a Small to medium sized deciduous tree. The Bark is dark grey, rough and corkey. The Leaves are spirally arranged, seldom clustered narrowly, lanceolate up to 15cm and dull green. *Boscia salicifolia* is known as zuoray in Northern Nigeria of West Africa. It belongs to the family *Capparacea* which are basically trees and shrubs. The tree with droop folix- like foliage attaining 13m height in dry savanna zone and commonly in association with termite and mound occurs from Senegal to Niger and Northern Nigeria and East Africa. The Distribution of the plant includes Ghana, Nigeria, Cameroon, Uganda, Kenya,

Tanzania, Malawi, Zambia, Bostwana and Zimbabwe.

Ethnomedicinal uses of *B. salicifolia* include treatment of nose bleeding, muscular pain and fatigue[3].

MATERIALS AND METHODS

Methanol, Petroleum ether, and distilled water, were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA). Extraction of the crude extract of *B. salicifolia* was carried out using methanol as solvent with Soxhlet apparatus. The extract was concentrated in-vacuo at 40^{o}C using rotary evaporator. The crude methanol extract was subjected to thin layer chromatography and vacuum liquid chromatography (VLC). FTIR analysis of the extract was carried out using FTIR-8400S Spectrometer system.

Gas Chromatographic/Mass Spectrometric analysis (GC-MS)

Preparation of extracts for GC-MS analysis:

The concentrated extract was re-dissolved in methanol, vortexed and filtered through 1ml syringe packed with cotton wool. 1µl aliquot of the sample solution was injected into a GC-MS (Model: QP2010 Plus Shimadzu, Tokyo, Japan) comprising a AOC-20i auto-sampler and gas-chromatograph interphased to a mass spectrometer (GC -MS) instrument equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70Ev was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.58 ml/min. Injector and mass transfer line temperature were set at 250 and 200°C respectively, and an injection volume of 1 µl was employed (split ratio 10:1). The oven temperature was programmed from 80°C (isothermal for 2 minute), with an increase of 9°C/min to 200°C for 4 minutes, 10°C/minute to 280°C, ending with a 5 minutes isothermal at 280°C. The MS operating parameters were, ionization energy, 70eV; ion source temperature, 200°C, solvent cut time, 2.5 minutes, relative detector gain mode, scan speed 1666 µ/second; scan range 40-800 u with interface temperature of 250°C. The total running time of GC-MS was 30 minutes. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of component

The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total areas. The detection employed the NIST Ver. 2.0-Year 2005 library. Interpretation of GC-MS was conducted using the database of NIST. The spectrum of the unknown phyto-components was compared with the spectrum of the known components stored in the NIST library. The name and molecular weights of the phyto-components of the test materials were ascertained.

RESULTS AND DISCUSION

The FTIR spectrum (Figure 1) was used to identify the functional group of the phyto-components based on the peak value in the region of infrared radiation as described by Roessner *et al*., (2001)[4] and Shellie *et al*., (2009)[5]. The FT-IR peak values and functional groups are presented in Table 1. The FT-IR confirmed the presence of alkanes, alkenes, alcohol, carboxylic acid and esters. Identification of phytochemicals by FT-IR analysis has also been reported by Sahaya *et al*., (2012)[6].

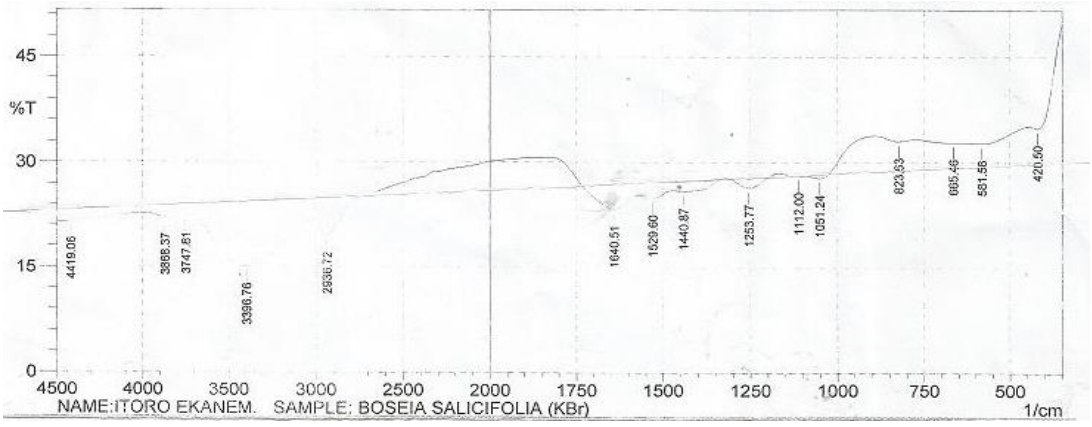

Figure 1: FT-IR spectral of methanol extract of B. salicifolia

Table 1: FT-IR peak values and functional groups of different phyto-compounds extracted from the methanol extract of *Boscia salicifolia*

Peak values (nm)	3396.76	2936.72	1640.51	1440.87	1253.77
Functional group	O-H Stretch H-bonded	C-H Stretch alkenes	C=C Stretch non-conjugated	C-C Stretch (in-ring)	C-O Stretch acid/Ester

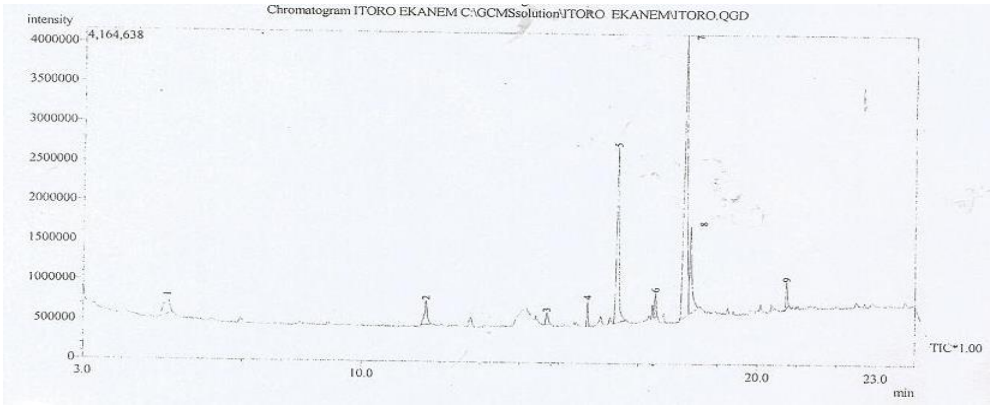

Figure 2: GC-MS of methanol extract of *B. salicifolia*

Table 2: Phyto-components identified in the methanol extract of *Boscia salicifolia* by GC-MS.

S/N	RT(min)	Name of compounds	Molecular Formula	Molecular weight	peak area (%)
1	5.13	1, 2, 3-propantriol	$C_3H_8O_3$	92	1.52
2	11.62	3, 5-di-tert-butylphenol	$C_{14}H_{22}O$	206	3.19
3	14.69	2-(2-hydroxy-2-phenylethoxy) phenol	$C_{14}H_{14}O_3$	230	0.62
4	15.73	Methy-12-methyltetradecanoate	$C_{16}H_{32}O_2$	256	0.90
5	16.48	n- Hexadecanioc acid	$C_{16}H_{32}O_2$	256	12.57
6	17.43	13-octadecanoate	$C_{18}H_{35}O_2$	283	5.61
7	18.18	9- octadecanioc acid	$C_{18}H_{34}O_2$	282	55.38
8	18.32	n- octadecanioc acid	$C_{18}H_{36}O_2$	284	14.74
9	20.73	9- octadecenal	$C_{18}H_{34}O$	266	5.47

RT: Retention Time

Table 3: Reported Biological activity of the identified active principles present in the methanol extract of *B. salicifolia*

Phytochemicals	Nature of compounds	Biological activities[7]
1, 2, 3-Propanetriol	Alcohol	Cleansing agent, a by-product in Soap manufacturing, Solvent, Cosmetics and lubricant.
n- Hexadecanioc acid	Fatty acid	Anti-alopecic, Aniti-androgenic, antioxidant, haemolytic, hyper-cholesterolemic, lubricant, nematicide, pesticide, propecic, flavor 5-alpha reductase inhibitor
13- Octadecanoate	Ester	Antiinflammatory, nematicide, hypocholesterolemic, Antiarthritic, Antieczemic agent
9- Octadecanioc acid	Fatty acid	Antiiflammatory, antialopecic, anemiagenic, 5- alpha reductase inhibitor, anti tumour, choleretic, dermatitigenic immunostimulant, anti-leucotriene-D4, antiandrogenic, lipoxygenic inhibitor, allergenic flavour, insectiguge, irritant, perfumery and propecic
n-Octadecanioc acid	Fatty acid	5-alpha reductase inhibitor, cosmetics, flavor, hypocholesterolemic, lubricant, perfumery propecic and suppository

The active phyto-components with their respective retention time (RT), molecular formula, molecular weight (MW), and relative percentages (peak area %) are listed in Table 2. Methanol extract of *B. salicifolia* contains 9-Octadecenoic acid (z) (55.38%), n-Hexadecanoic acid (12.57%), n-Octadecanoic acid (14.74%), 1,2,3-Propanetriol (1.52%), 3,5-di-tertbutylphenol (3.19%), 2-(2-hydroxy-2-phenyethoxy)phenol (0.62%), Methyl-12-methyltetradecanoate(0.90%), 13-Octadecanoate (5.61%), and 9- Octadecenal (5.47%). The biological activities of the identified phyto-components as reported in phytochemical and ethnobotanical database[7] are presented in Table 3.

CONCLUSION

Nine phyto-components have been identified from the methanol extract of *B.salicifolia* by GC-MS analysis. The results indicate the existence of various bioactive principles that justifies the uses of *B. salicifolia* for treating various ailments in traditional medicine. The results of this study represent the fingerprint and a biochemical marker of the plant. However, isolation and purification of individual phyto-component and studies of their biological activity would provide more information on the pharmaceutical and neutraceuticals potentials of *B. salicifolia*.

ACKNOWLEDGEMENT

The authors wish to thank Mallam Salmanu for plant identification, Mr. Victor Ochigbo and Mallam Mamoon Aboki of NARICT, Zaria, Nigeria for running the FTIR and GC-MS. The authors are also grateful to Kaduna Polytechnic for supporting the research.

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