

Isolation, Identification and Characterization of Daucosterol from Root of *Mangifera Indica*

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ABSTRACT

Medicinal plants are sources of therapeutic means for man several years ago till date. It has been of concern in research for new agents for treatment of both emerging and existing ailments as a result of shortcomings of existing synthetic drugs. In contributing to this search, this study aimed at isolating, identifying and characterising potential medicinal agents from root of *Mangifera indica*. The powdered plant part was extracted with hexane and ethyl acetate successively using soxhlet apparatus. These extracts were fractionated in silica gel column and using varying ratios of hexane and ethyl acetate and fractions obtained were observed using thin layer chromatography in different solvent systems of hexane, ethyl acetate and detecting reagents such as iodine vapour, UV-lamp and H₂S₀4. Based on the R_f values obtained from the TLC, the 50 fractions obtained were pooled to 5 and coded as NPJS 1-2, 3-4, 5-6, 7-8, 9-10 respectively. The colourless coded compounds were subjected to melting point determination and spectroscopic analysis (proton and carbon-NMR) as well as HMBC, HSQC and COSY. The result showed that NPJS 3-4 was a Daucosterol. The findings from this result indicates that Daucosterol has never been reported from this part of the plant.

Introduction

The use of plants by man is traceable to their usefulness in producing varieties of drugs, foods, as source of oxygen for man and animals, raw materials for many industries. Plants have proved to be the most useful in curing different diseases and are useful in pharmaceutical industries because of some chemical substances. These chemical substances include steroids, terpenes, flavonoids, alkaloids, tannins, glycosides, phenols, anthraquinones, carotenoids and saponins. These chemical substances are called phytochemicals or secondary metabolites [1]. Phytochemicals are biologically active substances that account for the medical ability of plants which serve as plant protective agents against various diseases and contribute to plants physical features such as aroma, colour and taste. The phytochemicals in plants serve as a protecting tool against environmental risks such as pathogenic attack, any forms of pollution, drought, UV exposure and enhance their usefulness in ethno medicine [2]. The study of plants has remained

the interest point over the years of many Phyto-Chemists. Though, the detection of useful clinical products from plant sources has been achieved partially and over 400 novel drugs recognized for viable use over 10 consecutive years, of which over 25 were new natural products and about 130 were improved natural products [3,4]. *Mangifera indica* is a member of group *Mangifera* and family Anacardiaceae, otherwise known as Mango family. It is made up of 30 genetic kind of the tropical edible fruit trees in the flowering plant from the family Anardiaceae with *Mangifera indica* been the most predominant class. *Mangifera indica* species has been described for their tradomedical uses for the management of syphilis, anemia, diabetes, diarrhea, ulcer, fever, and urinary tract infection [5]. Plant stigmastanes have been reported for their antitumor, antidiabetic and anti-inflammatory effects and cholesterol lowering [6]. This study, therefore, investigate the root of *Mangifera indica* with the aim of isolating, identify and characterizing bioactive compounds responsible for its ethno-medical uses.

Materials and Methods

Plant collection and Preparation

Roots of *Mangifera indica* were obtained in June, 2019 from Bunu Tai, Tai Local Government Area, Rivers State, Nigeria and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt and deposited at the Departmental Herbarium with voucher specimen No: UPH/2019/MIR/231. The root was kept clean with distilled water and dehydrated at room temperature for two weeks, crushed with the aid of a pestle and mortar and deposited in a tight basin, thereafter, moved to the laboratory for further experimentation.

Sample Extraction

Pulverised root of the sample (2kg) was serially extracted using soxhlet apparatus (n-hexane and ethyl acetate). Surplus solvent was a loof below reduced pressure to give unfinished residue extract labelled NPJS and kept in the refrigerator. The ethyl acetate extract was dissolved in n-hexane and chloroform (1:1) and reflux for three hours then filtered.

Chromatographic Separation

The filtrate was exposed to column chromatography via silica gel by means of stationary phase. The solvent system comprised of n-hexane in mixture with ethyl acetate. Approximately 30 mL fractions were taken by gradient elution procedure and elutes were observed using thin layer chromatography of 30:70 of ethyl

acetate and hexane solvent ratio. The fractions displaying related Rf value after envisioning by UV-lamp were assembled together. Collected fractions were confirmed with thin layer chromatography by spurting with anisaldehyde-sulphuric acid and the pink colour spots were established as stigmastanes. Fractions agreeing to Rf values were assembled, washed with acetone, and subjected to TLC with hexane-ethyl acetate 8:2 (v/v). Fractions NPJS 3-4 (23.4 mg) were discovered to have crystals which were independently washed and repeated with hexane (35 ml). TLC with hexane and dichloromethane (1:2) gave a distinct spot with Rf value 0.47. The purified fraction was labelled NPJS 3-4. This was established using Keller-kiliani reaction and Liebermann-Burchards test as described [7].

Spectroscopic Characterization

The purified coded fraction was subjected to melting point determination, IR, and spectroscopic analysis (Proton and Carbon-NMR) as well as HMBC, HSQC and COSY to explain the structure of the compound. The spectra ¹H, ¹³C, 2D NMR were verified with the aid of acetone as solvent on a Bruker Avance 3 spectrophotometer at University of Strathclyde, Glasgow, Scotland, United Kingdom.

Results and Discussion

The spectroscopic characterization result of NPJ 3-4 (Daucosterol) from *Mangifera indica* root are reported in (Tables 1&2) (Figure 1).

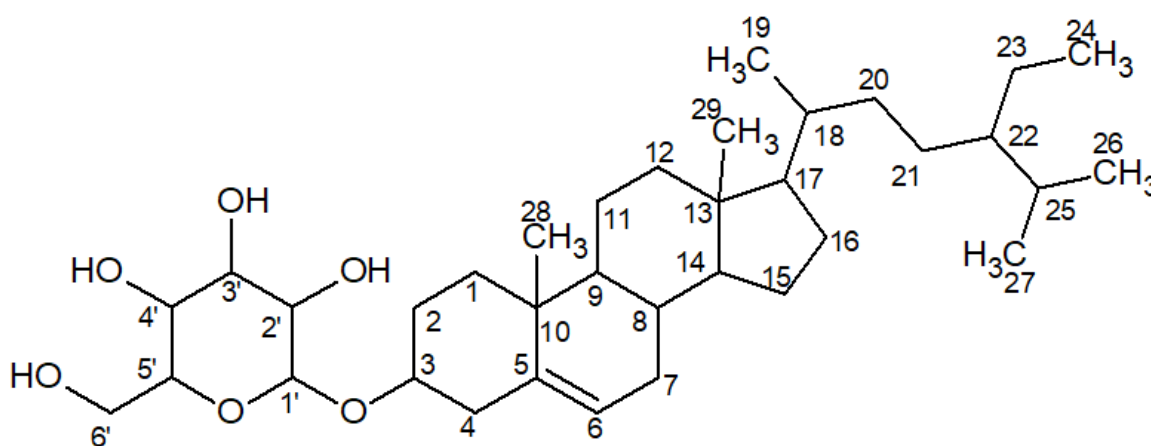


Figure 1: NPJS 3-4 as Daucosterol.

Table 1: ¹HNMR and ¹³C NMR chemical shifts for NPJS 3-4.

Spectroscopic technique	Data
1R(cm-1)	3450, 2850, 1095, 1379, 836
Rf	0.47
Mp	283-2860C
¹ HNMR(Acetone)	δ 1.00, 1.24, 2.88, 2.33, 5.30, 1.77, 0.91, 1.05, 1.24, 0.75, 4.32, 2.89, 3.28, 3.05, 3.28, 4.54 ppm
¹³ C NMR(Acetone)	δ 37.02, 29.6, 76.7, 40.3, 139.5, 121.4, 31.8, 29.6, 51.1, 34.6, 21.4, 40.3, 40.5, 55.7, 21.5, 28.6, 55.7, 12.6, 19.3, 37.0, 21.6, 34.5, 25.4, 45.6, 28.6, 19.3, 13.4, 21.5, 11.6, 101.3, 73.5, 76.7, 70.6, 76.8, 61.6 ppm

Table 2: Comparison of NMR data for NPJS: 3-4 with literature.

C-position	Experimental		Literature Peshim and Kar (2017).	
	¹ H(δ)	¹³ C(δ)	¹ H(δ)	¹³ C(δ)
1	1.00	37.02	1.00	36.79
2	1.24	29.6	1.26	29.23
3	2.88	76.7	2.98	76.85
4	2.33	40.3	2.26	39.28
5	-	139.5		140.4
6	5.30	121.4	5.35	121.2
7	1.77	31.8	1.73	31.38
8		29.6		31.26
9		51.1		49.55
10		34.6		36.18
11		21.44		20.56
12		40.3		38.26
13		40.5		41.82
14	0.91	55.7	0.95	56.13
15	1.05	21.5	1.05	23.83
16	1.24	28.6	1.25	27.76
17		55.7		55.38
18	0.75	12.6		11.64
19		19.3		19.07
20		37.0		35.45
21		21.4		18.58
22		34.5		33.29
23		25.4		25.36
24		45.6		45.09
25		28.6		28.64
26		19.3		19.69
27		13.4		18.89
28		21.5		22.06
29		11.6		11.75
1'	4.32	101.3	4.20	100.75
2'	2.89	73.5	2.89	73.42
3'	3.28	76.7	3.27	76.86
4'	3.05	70.6	3.00	70.04
5'	3.28	76.8	3.06	76.72
6'	4.54	61.6	4.58	62.82

Characterisation of compound NPJS 3-4 as Daucosterol

The fraction coded NPJS 3-4 was obtained from the ethyl acetate extract of *Mangifera indica* root as violet colour from sephadex column and gave an R_f value of 0.47 on thin layer chromatography. The IR spectrum of the compound showed a broad band in the region 3450 cm⁻¹ indicating presence of -OH of glucoside linkage [8]. The absorption observed at 2958-2850cm⁻¹ was allotted to asymmetric aliphatic -CH stretching of CH₃ and CH₂ groups. A band observed at 1095 cm⁻¹ was assigned to C-O-C linkage. The weak absorption

observed at 1664 cm⁻¹ was assigned to (C=C) stretching. However, three other bands were observed at 1452, 1379 and 836 cm⁻¹. The ¹H-NMR spectrum of compound NPJS 3-4 showed a chemical shift in the range δH 0.79-0.97 suggesting presence of methyl protons. A singlet observed at δH 0.75 was assigned to methyl protons. The proton attached to olefinic linkage was observed at δH 5.30. The signals observed at δH 0.79 and δH 0.91 were assigned to methyl groups of isopropenyl moiety (Peshin and Kar, 2017). Methyl proton was observed at δH 0.91 with coupling constant J=6.4Hz. The proton of glucose was observed at δH 2.88-4.32 as a multiplet.

The proton of CH-group of glucoside was observed at δH 4.40. Proton was observed at δH 2.88 the hydroxyl protons of the sugar moiety showed resonance at δH 3.56-3.63 [9].

The ^{13}C -NMR spectrum of the compound indicated 35 carbon signals of which six were for the sugar moiety and 29 attributed to the aglycone moiety. The carbon signals observed at δC 61.6 (C-6'), δC 70.6 (C-4'), δC 73.5 (C-2'), δC 76.8 (C-5'), δC 76.70 (C-3') and δC 101.3 (C-1') were well stable with those of glucose moiety (Lee et al; 2004). The aglycone moiety signals were observed at δC 139.5, δC 121.4, δC 76.7, δC 55.7, δC 55.7, δC 51.1, δC 45.6, δC 40.5, δC 40.3, δC 40.3, δC 37.02, δC 34.6, δC 36.0, δC 37.0, δC 34.5, δC 31.8, δC 29.6, δC 29.6, δC 28.6, δC 28.6, δC 25.4, δC 21.5, δC 21.5, δC 21.44, δC 19.3, δC 19.3, δC 13.4, δC 21.4, δC 11.6 and δC 12.6 (Table 1). The chemical shift at δC 19.3 and δC 21.4 were allotted to two methyl groups. The signals observed at downfield δC 12.6, δC 19.3 and δC 21.4 were given to angular methyl group moiety associated to C-18, C-19, and C-21. The signals at δC 139.5, δC 34.6 and δC 40.5 were assigned to the quaternary carbons at C-5, C-10, and C-13. Signals observed at δC 29.6, δC 51.1 and δC 55.7 were assigned to carbon at C-8, C-9, and C-14, respectively. An upfield chemical shifts at δC 37.02, δC 29.6, δC 40.3, δC 31.8, δC 51.1, δC 21.44, δC 40.3, δC 55.7, δC 21.5 ppm, δC 28.6 and δC 55.7 may be due to cyclohexyl and cyclopentyl of A, B, C and D-rings. Chemical shift at δC 37.0, δC 76.70 and δC 101.3 could be ascribed to carbon of cyclic linkage and anomeric carbon, respectively. The 1H and ^{13}C -NMR spectra data were in line with literature reports (Peshim and Kar, 2017) for a polyphenol with glucose and aglycone moieties, thus, fraction NPJS 3-4 was identified as stigmastane commonly called β -sitosterol-3-O- β -D-glucoside (Daucosterol) [10].

Conclusion

The isolated compound has been reported for its antibacterial and antifungal activities and thus could also serve as useful precursor in the synthesis of drugs to treat diseases caused by some clinical pathogens which justifies the ethno medical claims of this plant uses for treatment of some bacteria caused diseases.

However, this study is the first to prove the presence of Daucosterol in this part of the plant.

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