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ISOLATION OF QUERCETIN RHAMNOSIDE FROM *CHROZOPHORA SENEGALENSIS* LEAVES EXTRACT

BY

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ABSTRACT
The ethanol extract of leaves of Chrozophora senegalensis used in ethnomedicine to treat diarrhea, rheumatism, stomach ache, inflammation and venereal diseaseswas subjected to phytochemical studies using standard procedure which revealed the presence of tannins, saponins, carbohydrates and flavonoids. Chromatographic separation of n-butanol fraction using silica gel led to the isolation of a compound identified as Quercetin-3-rhamnoside.

Key Words: Traditional medicine, Quercetin-3-rhamnoside, medicinal plant, *Chrozophora senegalensis.*

INTRODUCTION

The term "traditional medicine" refers to ways of protecting and restoring health that existed before the arrival of modern medicine (WHO, 1996). As the term implies, these approaches to health belong to the traditions of each country, and have been handed down from generation to generation.

OBJECTIVES OF THE STUDY

The specific objectives are:

i. Collection and identification of the plant
ii. Extraction of the root portion of the plant.
iii. Preliminary phytochemical screening using the crude extract and the fractions.
iv. Fractionation of the crude extract.

LITERATURE REVIEW

Traditionally leaves of the plant called *Chrozophora senegalensis* (Damaigi in Hausa) are used as an astringent for diarrhea especially in children. This plant is mostly used for this purpose in northern part of Nigeria. The seed of this plant is used as contraceptive, treatment of syphilis and cataract (Bello, 2005).

Description of the plant

Chrozophora senegalensis belongs to the family of plants called Euphorbiaceae. The plant is a low under shrub, petioles short; leaves ovate-rhomboid. Flower (May-Dec.) Scarlet; fruits (May-Dec) Scaly Capsules (Irvire1961). In Mali *Chrozophora senegalensis* is used in folk medicine for the treatment of diarrhoea, rheumatism, teniasis stomach-ache, rachitic and venereal disease. Root and leaf decoctions are also drunk for hair loss (Vassallo *et al.*, 2006).
A Biflavonoid and amentoflavone were also isolated from *Chrozophora senegalensis* which was reported to have anti-tumour activity (Valeria, *et al.*, 2011).

Materials and Chemicals

All the chemicals used are of analar grade and were distilled before used. The silica gel for column chromatography is of mesh size (60-120), merk and that of thin layer chromatography is 0.25mm thickness, sephadex LH-20 (Sigma). Silica gel G, pre-coated Aluminium sheets (0.25mm) MERCK and Preparative TLC 0.5mm glass plate (MERCK), HNMR spectroscopy was carried out on Bruker DRX at 400MHZ 3.2.

METHODOLOGY

EXTRACTION PROCEDURES

(a) Collection and drying of plant material

The plant, *Chrozophora senegalensis* was collected at Kazaure local Government Area of Jigawa State and was authenticated by officials of the herbarium in the Department of Biological sciences, Ahmadu Bello University, Zaria Nigeria, and a herbarium sample was deposited. The plant material was dried in air under shade for one week. The leaves removed, crushed and then pounded into powder form.

(b) Extraction

200g of the powdered leaves of the plant was extracted to exhaustion using soxhlet with petroleum ether (60-80^{0}C), and the solvent evaporated to give a greenish oily mass (14.5g), (7.25%). The defatted marc was air –dried, extracted with 95% ethanol using soxhlet to exhaustion. The ethanol extract was concentrated at room temperature to give a greenish gummy mass (36g), (18%) coded ethanol extract similarly, 20g of powdered seed extracted

with petroleum ether to give 13g of the oil coded PEES, this was subjected to GC mass spectroscopy.

The ethanol extract was de-pigmented with activated charcoal.

20g of the crude ethanol extract was dissolved in water and filtered. The water-soluble part was extract with ethyl acetate (2x 500ml) and n-butanol (5x500ml) to give EA and NB

(c) Preliminary phytochemical screening
The crude ethanoic extract (EE), the petroleum ether soluble fraction (PE), and the n-butanol soluble fraction (NB) were subjected to preliminary phytochemical screening using standard procedure.

Test for carbohydrate

a. General test (molisch's test)

A few drops of the molisch's reagent was added to a clear solution of the extract dissolved in water followed by the addition of 1ml of conc. sulphuric acid down the side of the test-tube, so that the acid formed a layer beneath the aqueous layer. The mixture was allowed to stand for about 2 minutes and then diluted with 5ml of water. Formation of a red colour at the interphase of the two layers confirmed the presence of carbohydrates (Trease and Evans, 1983).

***(b)* Test for Sugars (Barfoed's Test)**
To a clear solution of the extract in water contained in a test tube, 1m1 of Barfored's reagent was added and heated on a water bath for 2 minutes, a red precipitate of Cu_2O was as a positive test tube for the presence of sugars (Brain and Turner, 1975).

(c) **Test for reducing sugars (Fehling's Test)**
The clear solution of the extract dissolved in water and heated with 5ml equal volumes of Fehling's solution A and B. formation of a red precipitate of CU_2O indicate the presence of reducing sugars (Trease and Evans, 1983).

Test for Alkaloids
9.5g each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with dragendorff's reagent. Turbidity or precipitate with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (Harborne, 1973, Trease and Evans, 1989).

Test for Tannin
About 0.5g eachportion of plant extract was stirred with 10ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue black, green or blue-green precipitate was taken as evidence for the presence of tannin (Trease and Evans, 1989).

Test for Saponins
The ability of saponins to produce frothing in aqueous solution was used as screening test for these compounds. For the frothing tests, the method described by Wall *et al.* (1952) was used. About 0.5g of each plant extract was taken with water in a test tube. frothing which persists on warning was taken as preliminary evidence for the presence of saponins.

Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of the plant was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Trease and Evans, 1989).

Test for Anthraquinones

Borntrager's test was used for the detection of anthraquinones. 0.5g of each plant extract was shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink red, or violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl anthraquinone (Trease and Evans, 1989).

For combined anthraquinone 0.5g of each plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. the filtrate was shaken with 5ml of benzene. The benzene layer separated and half its own volume of 10% ammonia solution added. A pink, red, or violet coloration in the ammonia phase (lower layer) indicated the presence of anthraquunone derivative in the extract (Trease and Evans, 1988).

Test for Flavoniods

a. Shinoda's Test

A little quantity of each of the extracts was dissolved in ethanol, warmed and filtered. three pieces of magnesium turnings were added to the filtrate followed by the addition of a few drops of conc. Hydrochloric acid; Orange, pink, red to purple colours will appear when flavones, flavonols, the corresponding 2,3-dihydroxy derivatives and / or xanthone are present (Silva *et al.*,1998).

(b) Ferric chloride Test

A little quantity of the extracts was dissolved in ethanol and boiled with a few drops of 10% $FeCl_3$ solution. A violet coloration was an indication of the presence of a phenolic hydroxyl group (Trease and Evans, 1983).

(c) Sulphuric Acid Test

Flavones and flavonols dissolve into concentrated Sulphuric acid giving a deep yellow solution. Chalcones and aurones produce red or red-bluish solution. Flavononeses give orange to red colors (Silva *et al.,* 1998).

Test for Cardiac Glycoside

a. Legal Test

The extract was dissolved in pyridine and few drops of 2% sodium nitroprusside together with a few drops of 20% NaOH were added. A deep red colourwhich faded to a brownish yellow indicated the presence of cardenolides (Silva et al.,1998).

b. Liebernan's Test

0.5g of the extract was dissolved in 2ml of acetic and cooled well in ice, H_2SO_4 was then carefully added. A colour change from violet to blue to green indicated the presence of steroidal nucleus (i.e. glycone portion of the cardiac glycoside).

c. Salkowski Test

0.5g of the extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (i.e., aglycone portion of the cardiac glycoside) (Silva *et al*., 1998).

Analytical Methods

Thin Layer Chromatography (TLC)

Ascending technique was used. the spots were applied manually using capillary tubes. the plates were developed at room temperature in an appropriate solvent system using a Shandon chromo tank. the developed chromatograms were dried in the air and visualized as follows:
(a) Viewed under sunlight
(b) Exposed to iodine vapour in a closed chamber

Thin Layer Chromatographic Examination of the n- Butanol Extract
Thin layer chromatography examination of the extract (100;16.5:.13.5) solvent system. Visualization of the chromatography using iodine vapour revealed the presence of four spots.

Column Chromatography (CC)

The gradient elevation technique was employed using sintered glass column (25cmx 1.5cm) parked by slurry Method. The adsorbent was silica gel (60-120mesh).

Separation of the component of n-butanol extract by column chromatography

2.5g of the extract (n-butanol) was applied over a well-packed silica gel column. It was eluted gradient with chloroform, then the polarity was gradually increased using methanol. The effluents were collected in 10ml aliquots and subjected to thin layer chromatography using solvent system I. Similar aliquots were pooled together, concentrated at room temperature.

Table A: Column Chromatography of n-butanol Extract

Effluents	**Ratio**	**Aliquots**	**No. of spots**	**TLC remark**
$CHCI_3$: Me OH	10.0	1-15	Four	1-15 combined
$CHCI_3$: Me OH	9.1	16-39	Four	16-35 combined
$CHCI_3$: Me OH	8.2	36-54	Three	36-54 combined
$CHCI_3$: Me OH	8.2	55-104	Three	55-104 combined
$CHCI_3$: Me OH	8.2	110-121	Two	110-121 combined
$CHCI_3$: Me OH	8.2	121-142	Three	121-142 combined

Separation of the components of fractions

105-109 (0.12g) was absorbed on a column packed with sephadex LH-20 and elute using methanol. The effluents were collected in 2ml aliquots and subjected to thin layer chromatography using solvent system I to afford a yellow amorphous solid coded F_I.

110-142 (0.5g) was absorbed on a column packed with sephadex LH-20 using methanol. The effluents were collected in 2ml aliquots and subjected to thin layer chromatography using solvent system I.

Purification of F_1

Compound F_1 was then purified on preparative thin layer chromatography using the solvent system ethyl acetate methanol: water (100:16.5.13.5) to give compound F_1 a yellow amorphous solid (3.5mg). TLC studies using the above solvent system showed a single spot.

NMR Spectroscopy of Compound F_1

HNMR spectroscopy was carried out on Bruker DRX 400MH_Z NMR spectrometer and was recorded in methanol (CD_3OD) and the spectra are summarized in table.

DESCRIPTION OF THE EXTACTS

(a) Petroleum ether soluble components

The petroleum ether soluble components coded P.E. on concentration gave a yellowish-dark thick gummy mass; which weighed 14g (7%).

The petroleum ether constituents which are soluble in ethyl acetate on concentration gave a dark gummy solid mass, 5 g (36%); this was coded "A".

The constituents in "A" which are soluble in methanol on concentration gave a green gummy mass weighing (1.15g or 23% (B).

(b) Ethanol Extract

The ethanol extract (EE) on concentration gave a greenish, thick gummy mass which weighed 36g (18%).

The ethyl acetate soluble part of the ethanol extract gave a greenish mass 1.5g (7.5%) and the n-butanol extract a yellowish-brown solid 2.5g (12.5%) coded NB.

Result of The Preliminary Phytochemical Screening

Preliminary phytochemical screening of the extracts revealed the presence of the plant constituents and shown below.

Table B: Result of the Preliminary Phytochemical Screening

CONSTITUENTS	TEST	OBSERVATION	P.E	E.A	ETH	n-B
Carbohydrate						
General	Molisch's	Red ring	-	-	+	_
Sugar	Barfoed's	Red ppt	-	-	+	_
Reducing Sugar	Fehling's	Red ppt	-	-	+	_
Alkaloid	Dragenorff's	Orange red ppt Buff ppts	- -	- -	_ _	_ _
Tannins	$FeCI_3$	Blue-Black, green or Blue green ppt	-	-	+	+
Saponins	Frothing	Frothing	-	-	+	_
Phlobatannins	1%HCI (aq)	Red ppt	-	+	_	_
Anthraquinones Combined Anthraquinones	Borntrager's	Pink,red or violet colours	_ -	_ _	_ _	_ _
Flavonoid	Shinoda's	Orange, pink, red, purple colour	_	_	_	_
	$FeCI_3$	Violet colour	-	-	_	+
	H_2SO_4	Deep yellow or red-bluish or orange-red	+	+	+	+
		Colour				
Cardia Glycosides	Legal	Deep red colour faded to brownish yellow	_	_	+	
	Libermaan's	Colour change from violet to blue to green		+	+	_
	Salkowski	Reddish-brown ring	+	+	+	-

Key: _ : Absent + : Present

Table C: TLC Properties of n-butanol

Spot	Visualization in I_2vapour	Rf value
a	Dark yellow	0.0
B	Dark yellow	0.3
C	Dark yellow	0.4
D	Dark yellow	0.6
E	Dark yellow	0.7

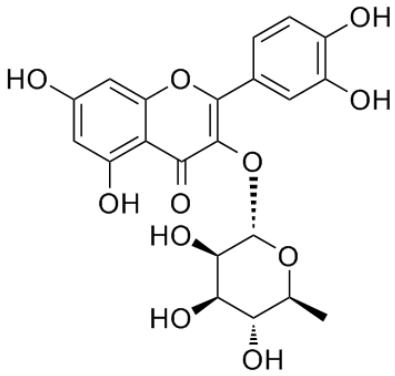
The TLC (ethyl acetate: methanol: H_2O 100: 16.5:13.5) chromatography of fraction 4 and 5 obtained using sephadex LH-20 to separate fraction 105-109.

Analysis of Compounds F_2

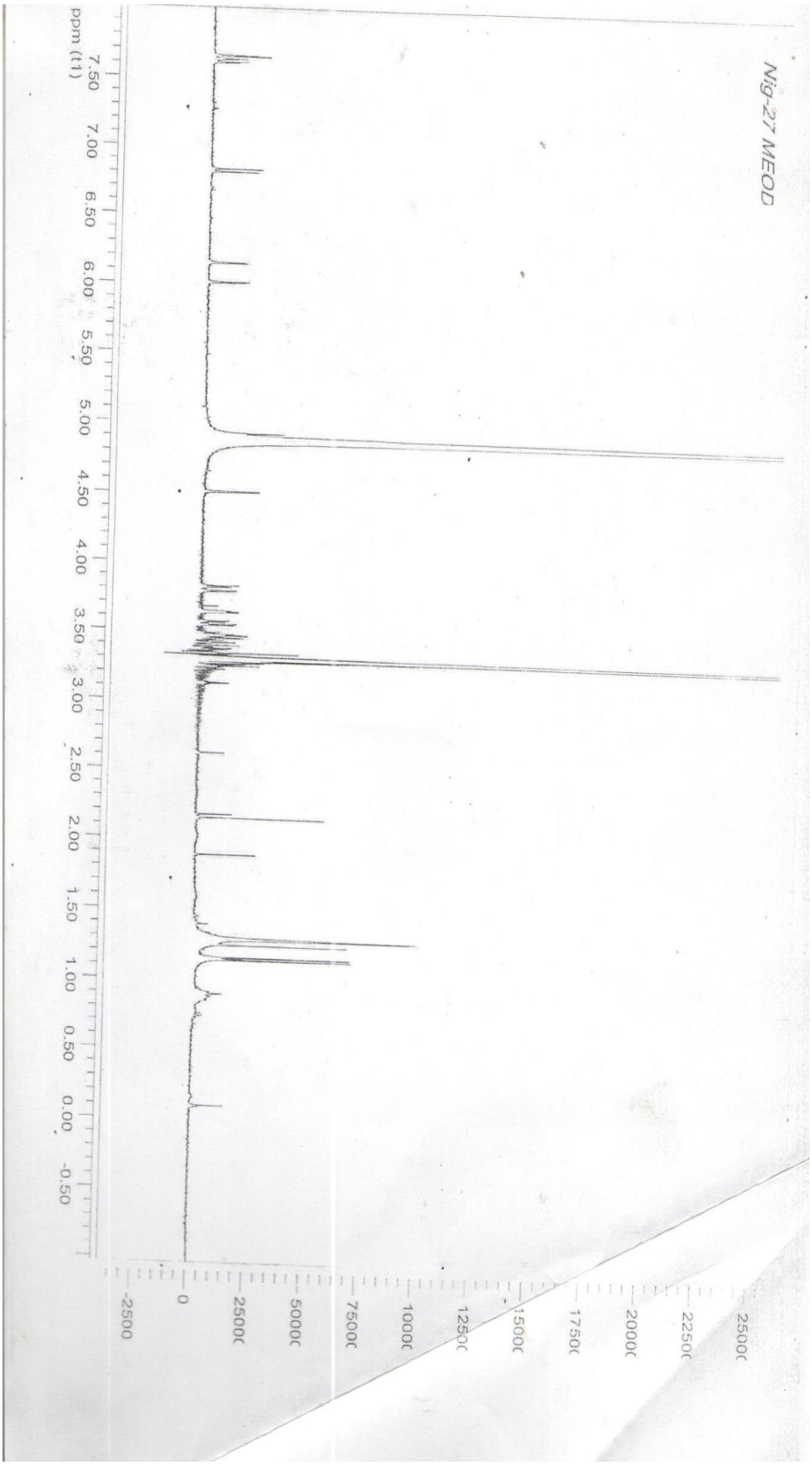
Compound F_2 was isolated as a yellow amorphous powder.[1] H-NMR spectrum suggested a flavonoids skeleton. The ^{1}H-NMR spectrum (table) indicates a 5,7- dihydroxylated pattern for ring A. (two meta coupled doublets at δ 6.02 and 6.15, J=2HZ) and a 3^1,4^1-dihydroxylation pattern for ring B (Signal at δ 6.80d, J = 8HZ; 7.65, DD, J = 2,8 HZ: 7.68 D, J = 2HZ) suggesting a quercetine nucleus. The 1H-NMR spectrums also showed signal ascribable to sugar moieties. An anomeric proton arising from the sugar moieties appeared at J 4.50 (^{1}H, S).

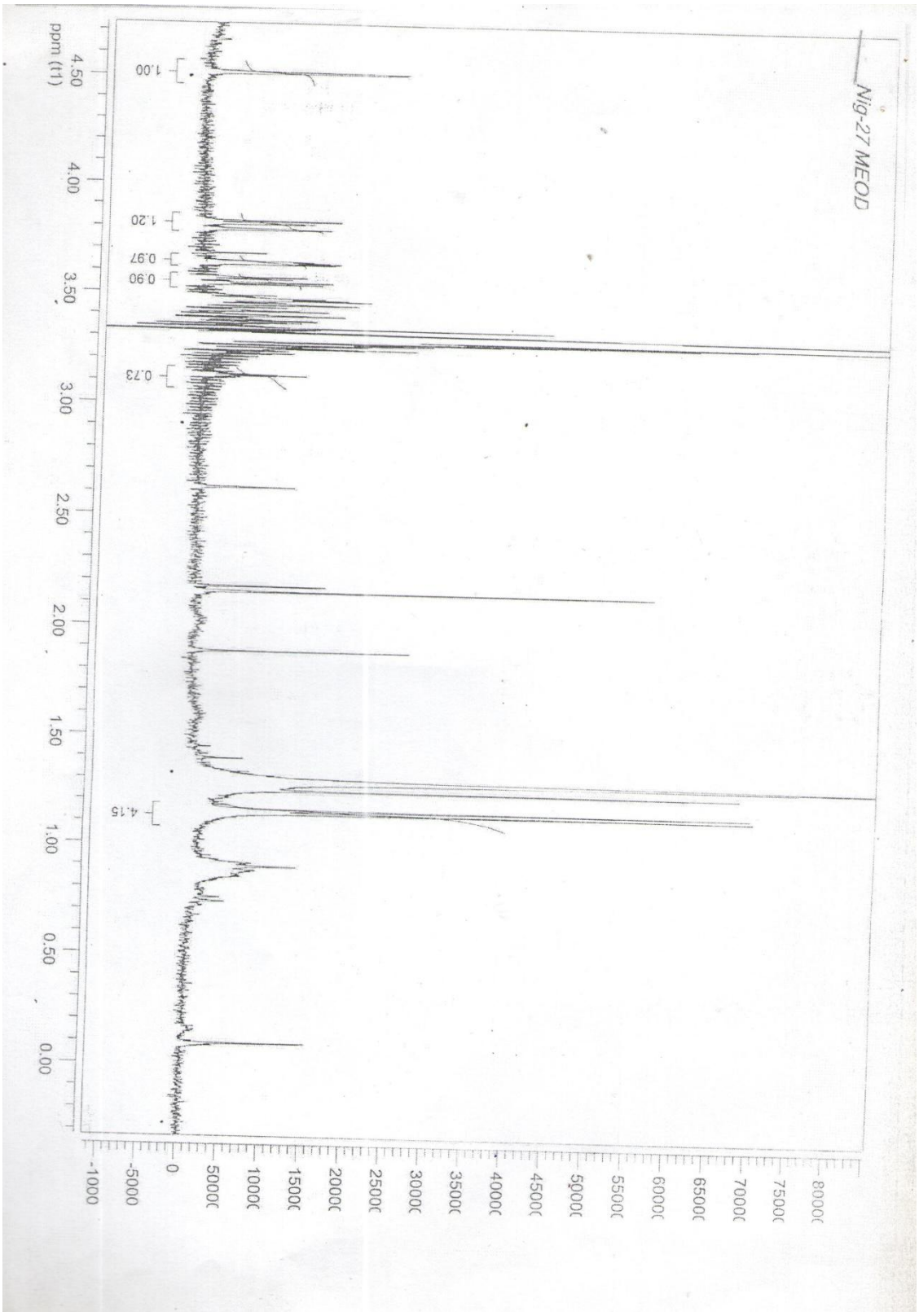
The ^{1}H-NMR spectrums also showed signals to sugar protons (δ 3.10, 3.60, 3.65, 3.85 and 4.50 PPM).

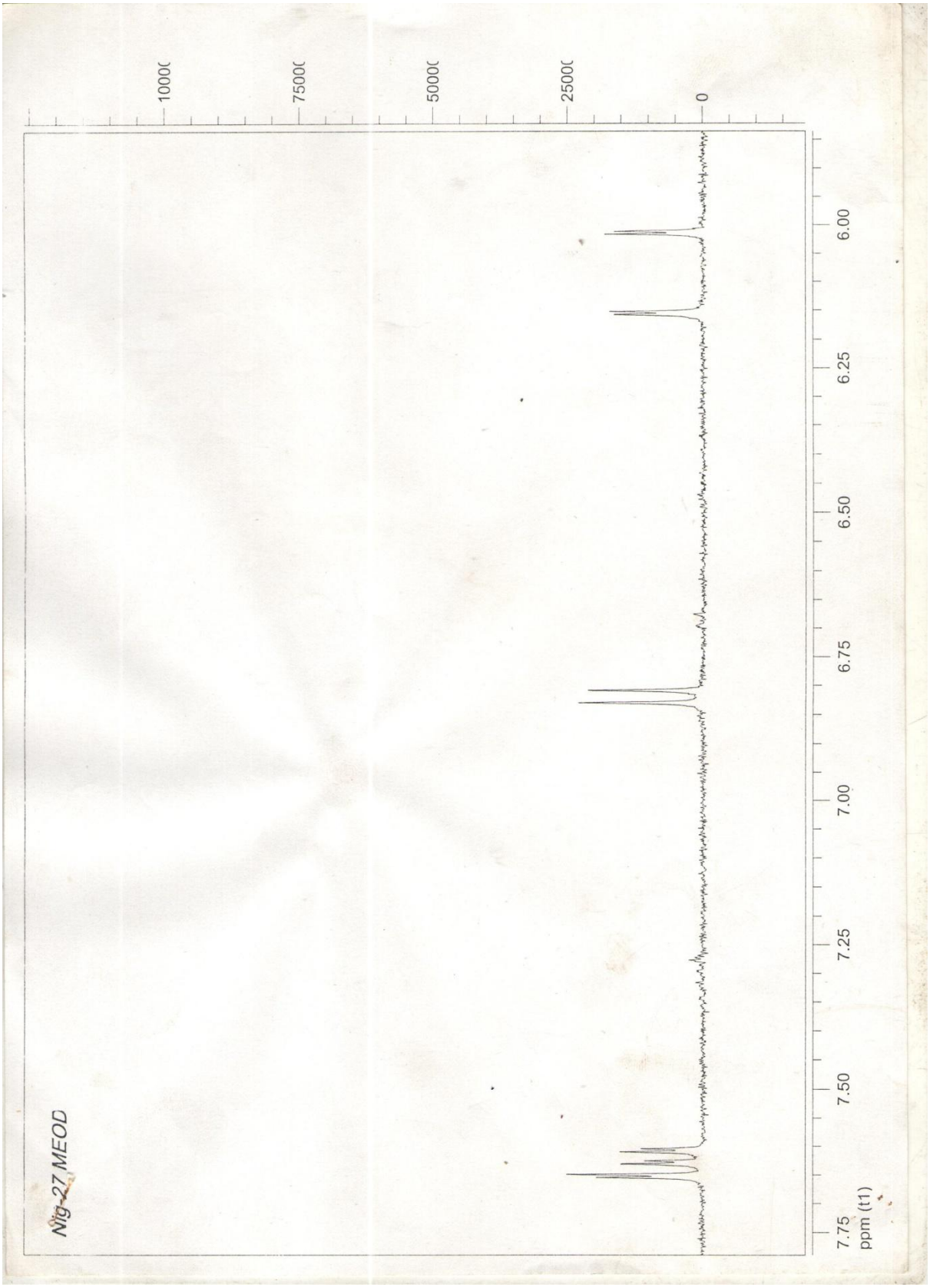
Therefore, the structure of the compound may be: -

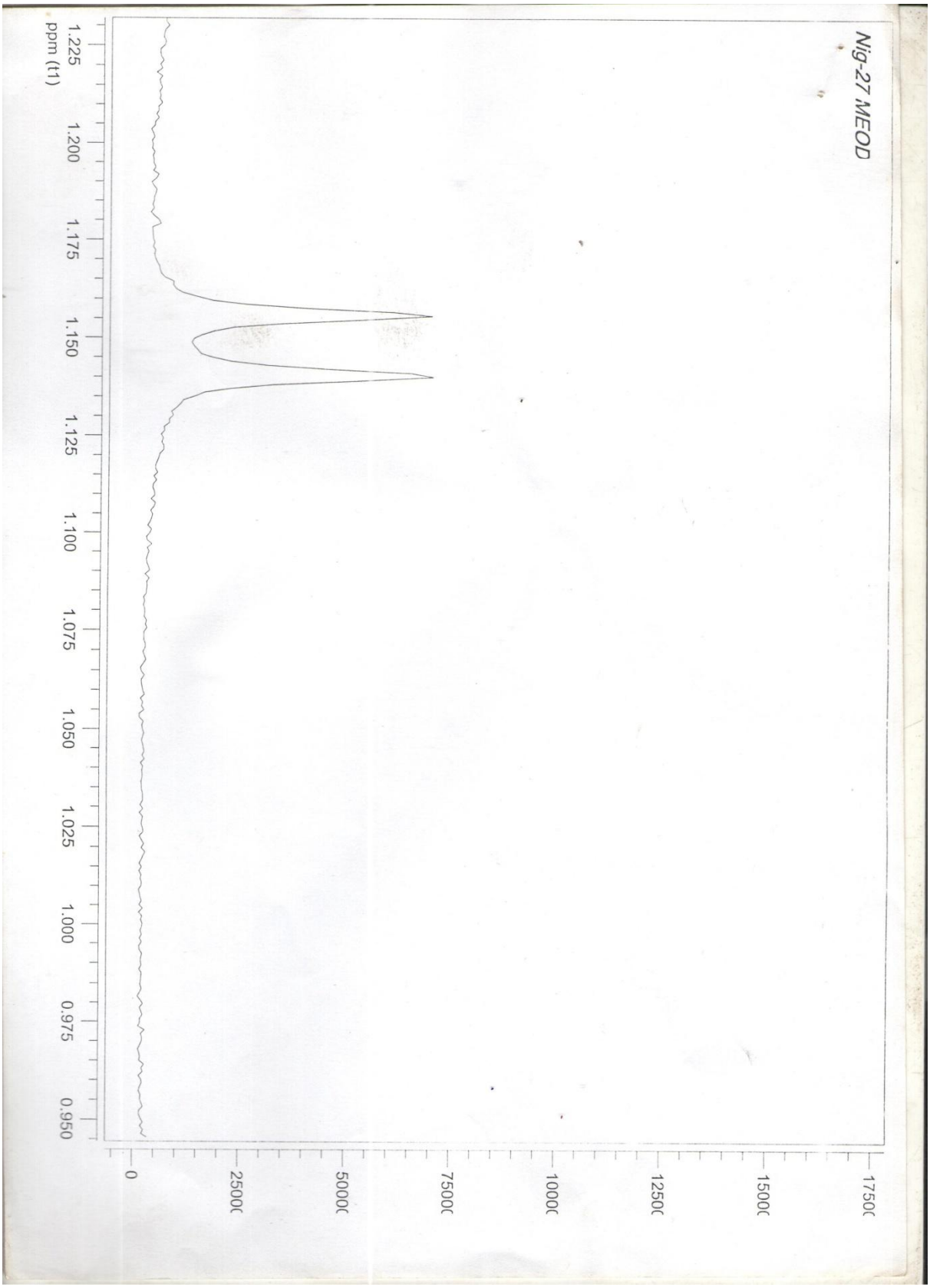


Quercetin 3 - rhamnoside




Nig-27 MEOD
ppm (t1)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1.00
1.20
0.97
0.90
0.73
4.15
-1000
-5000
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
55000
60000
65000
70000
75000
80000


10000
7500
5000
2500
0
6.00
6.25
6.50
6.75
7.00
7.25
7.50
7.75
ppm (t1)
Nig-27 MEOD


Nig-27 MEOD
ppm (t1)
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1.200
1.175
1.150
1.125
1.100
1.075
1.050
1.025
1.000
0.975
0.950
0
25000
50000
75000
100000
125000
150000
175000

DISCUSSION

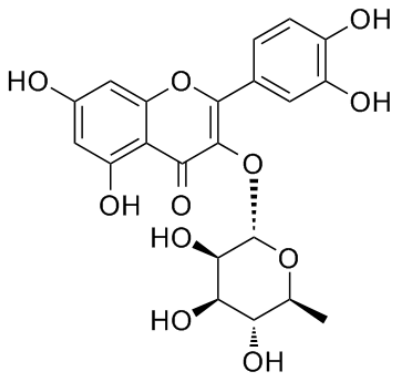
The preliminary phytochemical investigation carried out on the leaves of *Chrozophora senegalensis* revealed the presence of saponins, flavonoids and Tannins. These metabolites have been shown to be responsible for therapeutic activity of plant (Trease and Evans, 1996).

Fractionation of the n-butanol soluble solution extract over silica gel G column chromatography and subsequent purification over sephadex LH-20 (methanol) and preparative TLC led to the isolation of compound F2 (3.5mg). Chemical studies (Shinoda) and $FeCI_3$ gave positive test for flavonoids (Harbone, 1975).

Compound F_2 was isolated as yellow amorphous powder. The ^{1}H-NMR spectrum (Table) indicates a 5,7-dihydroxylated powder. For ring A. (two meta coupled doublets at δ 6.02 and 6.15, J =2HZ) and a 3^1, 4^1) dihydroxylation pattern for ring B (Signals at δ 6.80d, J = 8HZ; 7.65, dd, J =2,8HZ: 7.68 d, J = $2H_Z$) suggesting a quercetin nucleus. The ^{1}H-NMR spectrum also showed signal assigned to sugar moieties in the range (δ = 3- 4.5PPM). The anomeric proton showed a singlet at 4.50 and methyl protons at 1.15PPM.

The ^{1}H-NMR spectrums also showed signals ascribable to sugar protons (δ 3.10, 3.60, 3.65, 3.85). Based on the ^{1}H-NMR spectra and that reported in literature (Harbone, 1975), compound F was found to be quercetin-3-L-Rhamnoside

Therefore, the structure of the compound may be



Quercetin 3-rhamnoside

Flavonoids are currently of growing interest owing to their supposed properties in promoting health (Rauha et al, 2000).

REFERENCES

Bello, M (2005): Personal communication

Brain, KR; Turner, TD; (1975): Extraction procedure. The practical evaluation of phytopharmaceuticals. Wright scent clinical, Bristol U.K. pp. 98.

Harbone, JB (1973): Phytochemical method. A guide to Modern techniques of plant analysis, Chapman and Hall, Pbls chapter 1. London. Pg. 7.

Irvin, FR (1961): Woody plants of Ghana. An oxford University Press publication, London. Pp. 218 – 219.

Silva, GL; Chai, H; Farnsworth, NR and Gupta, MP (1998): In natural products isolation. Edited by Richard Carnell, A. Humana press publication, New Jersey. Pp. 349 – 359.

Trease, GE and Evans, WC (1983): A textbook of Pharmacognosy, 12th ed. Bailliere Tinda, London. Pp. 658 – 682.