IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736. Volume 5, Issue 6 (Nov. – Dec. 2013), PP 51-54
www.iosrjournals.org

Isolation and Characterization of Colchicine from Physostigma Venenosium

[1]Ikpa C. B.C and [2]Johnbull E. O
[1]*Dept. Of Chemistry, Imo State University Owerri Imo State Nigeria*
[2]*Dept. Of Chemistry Mickeal Okpara University Of Agriculture Umudike Abia State Nigeria*

Abstract: *A unique anti-inflammatory drug Colchicine has been isolated from a Nigeria medicinal plant called Physostigma venenosium The structure was established using NMR spectroscopy of ^{1}H and ^{13}C, Cosy, and DEPT In combination with IR. The ethanol extract of the plant seed was partitioned to get the chloroform, water, methanol and pet-either fractions. The chloroform fraction, from which colchicines was isolated was discovered to contain the highest number of compounds with wide range of R_f values on TLC result.. The result supports the use of the plant seeds for the treatment of phantom tumor and conjunctival inflammation.*
Key words*: anti-inflammatory, phantom tumor, physostigma venenosum, isolation.*

I. Introduction

The search for potent anti-infective agents occupies an important position in science more so with the upsurge in disease diversity and declining sensitivity of the implicated organisms available agents [1-2]

Medicinal plants are proven sourced of bioactive compounds against wide spectrum of diseases and infections [3-6].

Nigeria is richly endowed with indigenous plants which are used in herbal medicine that cure diseases and heal injuries. Some of these plants serve as food, medicine and poison. These plants exhibit wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, laxative, anti-spasmodic, anti-hypertensive, anti-diabetic and anti-microbial functions [7].

A unique anti-inflammatory drug colchicine has been isolated from a Nigerian medicinal plant called PhysostIgma venenosum, (esere bean or calabar bohm) [8].

The plant Physostigma venenosum is confirmed to have secondary metabolites of high anti-cholinesterase activity that gave a very good comparison with Huperzin
'A' – a synthetic anti-cholinesterase agent [9]

Physostigma venenosum is used to reduce tension caused by irritation, use to treat epilepsy, convulsion, inflammation, tumor, glaucoma, ulcer, chronic constipation etc [10].

Apart from being use to treat diseases it is locally used as ordeal poison in trial of witchcraft [11]

The purpose of this paper is to ascertain the use of Physostigma venenous an ordeal poison to treat diseases like tumor, inflammation, ulcer etc. The potential of physostigma venenosum as a drug and strong anti-cholinesterase agent (9) has been shown in isolation and characterization of sagainarine N diglycoside (9), phyysostigmine , digitalis[12] and comparison of the enzyme assay of the extract with Huperzin "A" [9]. The study involves the isolation, characterization and TLC evaluation of possible number of compounds soluble in the partitioned fractions of the crude extract.

II. Materials And Methods

Plant Materials

The seeds of physostigma venenosum were bought from Ariria International Market Aba Abia State on 14th July 2006. The seeds were identified by Dr. A. Nmeregini of the Forest Department, University of Agriculture Umudike Umuahia Abia State of Nigeria.

Extraction And Isolation Of Plant Materials.

Peeled seeds sample weighing 4.12kg were dried at room temperature. The dry seed sample was grounded to powdery form and 3.12kg was percolated with 4 litres of redistilled ethanol for one week. The extract was filtered and concentrated with rota evaporator at 35^0c.

The concentrated ethanol extract F^0 was dried to obtain a dark brown gummy crude extract (31.86g). A portion of 26.33g of crude extract was partitioned between chloroform and water to obtain 8.97g of chloroform fraction ($1F^A$). 4.41g of the chloroform fraction was then partitioned between petroleum ether ($60 – 80^0$c) and aqueous methanol to obtain 2.13g of methanol fraction ($2F^A$) and 2.27g of pet ether fraction ($2F^B$). The possible number of compounds present in each partitions crude fraction was evaluated using TLC. The TLC was carried out in

Chemistry Laboratory of Michael Okpara University of Agriculture Umudike. Silica gel of Merck grade 60a (30g) and distilled water (100mls) were used for making slurry.

The homogenous mixture of the slurry was used to coat the TLC plate (glass of 5 x 15cm). The evenly coated plates were allowed to dry before taken to oven for activation. The solution of the test sample fractions were made with their solvent medium. Sports of the test sample (2 or 3) were dropped on the plate and dipped into TLC tank containing solvent mixture of different polarity. The plates were removed from the tank after 75% of solvent movement. TLC plates were allowed to dry before developed in an iodine tank containing iodine crystals. The developed sports which were yellow, brown or dark brown in colours were measure with ruler to obtain the retention factor (R_f).

R_f = movement of sample / Movement of solvent front (13)

4.16g of the chloroform fraction was subjected to column chromatography over silica gel Merck grade, (60 – 120 mesh).The eluted fractions labeled in roman numerals were analysed with TLC silica gel Merck grade 60A with gypsum binder

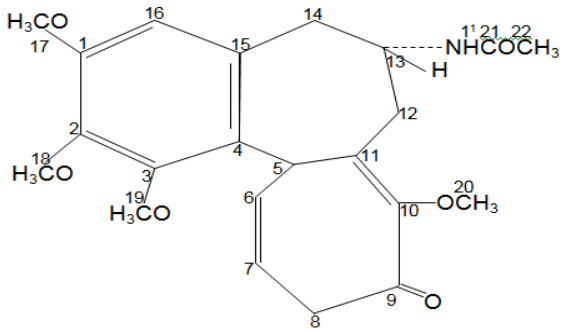
The yellow sticky fraction ix labeled **Ik – A** (0. 15g) gave one spot on the TLC with R_f (0.67).The pure compound IK – A was further analysed to be Colchicine using IR PerkinElmer model 760. The mass was determined with HREIMS positive ion mode. Brucker multinuclear NMR experiments of ^{1}H and ^{13}C, cossy of ^{1}H – ^{1}H and ^{13}C DEPT 135 and DEPT 90 of ^{13}C NMR recorded at **F1** 300MHZ and **F2** 300MHZ using TMS as internal standard were used. Ethanol, methanol-, petroleum-ether (pet ether), chloroform, ethyl acetate, acetone, DMSO and iodine crystals were procured from Merck.

III. Results & Discussions

The crude extract which was (ethanol extract) yielded 0.99% of the dry powdered seeds sample. Cold extraction was used in place of hot extraction methods like soxhlet to avoid loss of volatile compounds in the sample. Water fraction was not used because of the attack of micro-organisms after 48 hours. The partitioned fractions which were labeled as ethanol= F^{o}; $CHCL_3$ = IF^{A}, H_2o = $1f^{B;}$ (aq) methanol =$2F^{A}$ and pet ether = $2F^{B}$ produce the following percentage yield when dried: - f^{0} (0.999%), $1f^{A}$ (33.69%) $2f^{A}$ (4.8.30%) and $2f^{B}$ (51.70%). The percentage yield of $CHCl_3$ ($1f^{A}$) was the smallest when compared with others, indicting the solubility of the component compounds in the above solvents used [13]. TLC result of the fractions in different solvent polarity showed the possible number of compounds in each fraction. TLC of **$1f^{A}$** in 50 -50% of $CHCl_3$ and pet-ether mixture eluted six compounds numbered A to F with the retention values as follows: A(R_f 0.18), B(R_f 0.34), C(R_f 0.41), D(R_f 0.51), E(R_f 0.79), and F(R_f 0.86). With 30% CHCl3 and 70% pet-either three compound were eluted; A(R_f 051), B(R_f0.87) and C(R_f18.94).

$2F^{A:}$ With equal mixture of solvent polarity of 50% $CHCl_3$ and 50% pet-ether all the compounds moved with the solvent front, while at 20% $CHCl_3$ and 80% pet ether there was no compound movement, 30% $CHCL_3$ and 70% pet-ether produced two compounds; A(R_f 0.71) and B(R_f 0.88).; **$2f^{B;}$** ,All the compounds moved with the solvent front at 50% $CHCl_3$ and 50% pet-ether, three compounds were observed with 30% $CHCl_3$ and 70% pet- ether A(R_{lf} 0.18), B(R_f 0.71) and C(R_f 0.88) while 20% $CHCL_3$ and 80% pet-ether showed no compound movement. The TLC result of the fractions showed that $CHCl_3$ (lf^{A}) contains the highest number of compounds with wide range of R_f values. The chloroform fraction If^{A} was further fractionated with column chromatography. One of the pure compounds (ix) eluted and labeled IK-A was further analyzed as Colchicine.

Fig1:



Compound ix labeled Ik-A was a yellow sticky substance with R_f of 0.56 on TLC. Its mass 412 was determined by M/Z of 412.051 and calculated for $C_{22}H_{27}O_6N$. The IR spectrum measured in cm^{-1} exhibited absorptions at 3407.1 for amide group, 2253.9 for (N-C), 2128.7 for (C=C-O) stretching, 16531.1 (C=O), 1049.1 to 1020 for (C-O-CH_3). Stretching, 760.7 for (C-H) adjacent aromatic rocking, 517.4 for (C-H_2) twisting while 823.0 CM^{-1}showed Para substituted benzene ring (table 1).

TABLE I; IR DATA OF COMPOUND Ik-A IR in CM^{-1} FUNCTIONAL GROUPS

3407.1	-CONH
2253.9	N-C
2128.7	C=C-O
1652.1	C=O
1049 – 1020	C-O-CH_3
823.0	Para substituted benzene
760.7	C-H (adjacent aromatic rocking)
517.4	C-H_2 (twisting)

IH NMR; Absorptions was observed at [δH; $5.621_{(s)}$, $6.911_{(s)}$ $5.606_{(s)}$ and $1.976_{(t))}$] with integral values of one hydrogen each indicating Methine protons, Saturated C-H proton with N- linkage at $\delta 7.282_{(q)}$ N-H proton at $\delta 7.485_{(s)}$ Methylene protons at, [δ; $4.451_{(d)}$, $22.970_{(d)}$ $4.188_{(d)}$] with integral values of two H each, Methoxy protons at [δ;$0.873_{(t)}$, $1.249_{(t)}$, $1.411_{(t)}$ and $1.593_{(t))}$], methyl proton with Nitrogen linkage at [$\delta 1.976_{(t)}$] all with integral values of three H each.

^{13}C NMR showed aromatic Qauntinary carbon at [δC; 128.55, 130.68 , 132.19, 136.50, 148.80, 152.87 and 155.87], carbonyl carbons (C=O) at [δC: 167.49 and 229.95] methine carbon at [δC; 127.80, 116.14, 107.20, 120.96, 120.24]. Signals of methylene carbon were shown at [δC;52.87, 67.90, and 96.67] Methoxy carbon were shown at [δc: 10.72, 13.80, 22.42, and 23.51] while methyl carbon appeared at δC; 30.12. (table 2))

COSY spectra showed coupling at H=5 and H=6 with (δH; $5.626_{(s)}$ and $6.911_{(s)}$,], H= 13 and H=1^1 [δH;. 7,282 $_{(q)}$ and $7.485_{(s)}$] and self coupling was observed at H =14 [δH; 4.188 $_{(s)}$].

DEPT 135 of 13C NMR showed the methoxy carbons of the benzyl ring at positive peaks, The methoxy carbon of the heptanyl ring and aliphatic methyl carbon appeared at the negative peaks. DEPT 90 showed only the methine carbons at the positive peaks. Combining the mass, IR, NMR of ^{1}H, ^{13}C, Cosy and DEPT, compound IK-A was identified as **Colchicine** with molecular formula of **$C_{22}H_{27}O_6N$.**

Colchicine was documented as a unique anti-inflammatory drug, unique because of its dramatic relief for acute gouty arthritis (14). It is also use as an anti-mitotic agent in cell division ($_{15}$).This result supports the use of the seeds of physostigma venenosum for the treatment of phantom tumor and conjunctival inflammation (10)

Table 2: ^{1}h And ^{13}c Nmr Data Of Compound 1k– A

ASSIGN-MENT	$\delta 13C$	MULTIP-LICITY	δ^1H	MULTIP-LICITY	INTIGRAL VALUE
1.	136.50	s			
2.	148.80	s			
3.	132'19	s			
4.	155.87	s			
5.	107.20	d	5.626	s	1H
6.	120.24	d	6.911	s	1H
7.	120.96	d	6.325	d	1H
8.	96.65	t	4.451	d	2H
9.	167.49	s			
10.	152.87	s			
11.	128.55	s			
12.	67.90	t	2.970	d	2H
13.	127.80	d	7.282	q	1H
14.	52.87	t	4.188	d	2H
15.	130.68	s			
16.	116.14	d	5.606	S	1H
17.	10.72	q	0.873	t	3H
18.	13.80	q	1.249	t	3H
19.	22.42	q	1.411	t	3H
20.	23.51	q	1.593	t	3H
21.	229.97	s			
22.	30.12	q	1.976	t	3H
1^1			7.485	s	1H

$C_{22}H_{27}O_6N$

References

[1.] S. N. Benerjee and T. G. Emori (1991): Am. J. Med. 91 (Suppl. 3B), 865 – 895.

[2.] C. N. Beck – Sauga and T. R. Jarvis (1991) . J. Infect, Dis. 167, 1247 – 1251.
[3.] C. M. C. Chariandy (1999); Screening Medicinal Plants from Trinidad and Tobago for Anti-microbial and Insecticidal Properties. Journal Ethno Pharmacology 64(3): 265 – 270.
[4.] D. E. Okwu, V. Ezenagu (2008): Evaluation of the Phytochemical composition of mango (Mangifera Indica Linn). Sten Back and Leaves. Int. Journal Chan and Sci 6(2): 705 – 710.
[5.] D.E. Okwu and O. N. Ohanhen (2009); Isolation, Characterization and Antibacterial activity of Lanostan Trilerperiod from the leaves of Stachytarpheta. (Jamaricensis Linn Vahl). J. Der Pharns. Chemica, 1(2): 32 – 29.
[6.] M. J. Sanz (1994): Influence of a series of Natural Flavouoids on free Radical Generating Systems and Oxidative stress Xenobiotlca 24 (7). 689 -699
[7.] D. E. Okwu (2007). Nigerian Medicinal Plants 1, J. of Medicinal and Arounatic Plant Science and Biotechnology 90 – 96.
[8.] Rehm S. (994): Multilingual Dictionary of Agronomic Plants (Rehm Dictionary) CRC Press USA p. 2064.
[9.] Johabull E. O. and Ikpa, C.B.C. (2013): Isolation, Characterization and Anti – cholinesterase activities of Physostigma Venenosum (Calabar Bean) Am. J. Scientific and Ind. Research (AJSIR) 4(2). 226 – 230.
[10.] Finely Ellingwood N.M.D (1919) by America material medical, therapeutic and Pharmacognosy.
[11.] Laura Spinney (2003): Histories, New Scientist Encyclopeadia Britannica. 11th Edition "The killer Bean of Calabar" p. 482.
[12.] Koelle G. B.(1995): Drugs acting at synaptic and Neuro Effector Functional site in Goodman L. S Gilman A. (Edition). The pharmacological basis of Therapeutics 5th Ed. New York, Macmillan Publishing Co. Inc. 404 – 476,
[13.] James A More David L. Oscar, R. Rodig (1983) Experimental Methods in Organic Chemistry 3rd Edition Holt Saunders International Edition p. 38 – 52.
[14.] Louis S. Goodman and Alfred Gilman (1997): the Pharmacological basis of therapeutics 5th Edition. Macmillan Publishing Co. Inc. New York p. 445 – 465.
[15.] Amoroso E. C (1935): Colchicine and Tumour growth Nature Land 135, 266 – 267.