Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.4, 2014

Isolation and Characterization of 5,7 – dimethoxy, 4'– propoxyflavone from the Seeds of *Mucuna pruriens* (Utilis).

Rosemary Izunwanne Uchegbu[1]*, JohnBull Onyekachi Echeme [2] and Irenus Chinonye Iwu [3]
*1. Department of Chemistry, Alvan Ikoku Federal College of Education,
P. O. Box 1033, Owerri. Imo State. Nigeria.
2. Department of Chemistry, Michael Okpara University of Agriculture Umudike. Umuahia . Abia State. Nigeria.
3. Department of Chemistry, Federal University of Technology, Owerri. Imo State. Nigeria
*Corresponding author, mrsriu@yahoo.com.

Abstract
Chemical investigation of the bioactive constituents of the seeds of *Mucuna pruriens* (Utilis) resulted in the isolation of a new flavone, 5,7 – dimethoxy, 4'– propoxyflavone. The structure was elucidated using NMR spectroscopy in combination with IR and MS spectra data.
Keywords: antioxidant, anti-inflammatory, flavone.

1.0 Introduction

Mucuna pruriens popularly known as "agbara" by the Igbo tribe of Nigeria is indigenous to India as a popular food crop and medicinal plant and also found in West Africa and Central America. It is an annual vine that grows up to 30ft. The leaves are alternate with three large rhomboid ovate leaflets. The flowers are large white to dark purple colour. It produces clusters of pods that are curved with silky hairs, each pod containing 2 – 6 seeds (Vadivel, 2010).
M. pruriens is extensively used in herbal medicine and as food. The seeds have been found to contain high levels of protein and carbohydrate and thus the flour has been recommended to be incorporated into cereals and root/tuber food products to improve their nutritional quality (Udensi and Eke, 2000). The flour is used in soup as soup thickener and to enhance the flavour of the soup. *M. pruriens* is grown widely as a green vegetable, both the green pods and the mature beans are boiled and eaten as food (Burkill, (1995); Duke, (1981)). The seeds are also roasted and ground to make a coffee substitute. Extracts from the seeds have been recommended as one of the best tonics to improve vitality (Manyam et al, 2004). The possibility of utilizing the seed as a source of industrial starch has been investigated in Brazil and results indicated that a starch with a high viscosity and suitable as a thickening agent for food product were obtained (Buckles et al, 1998).

M. pruriens is highly valued because of its medicinal use. It is used in herbal medicine as a powerful nervine tonic and an effective aphrodisiac in the treatment of disorders of the male and female reproductive systems (Numida, 2009; Ezueh, 1997; Amin et al, 1996). The seeds are effective in the treatment of Parkinson diseases (Manyam et al, 2004; Katzenschlager et al, 2004). Extracts from the seeds have been used in herbal medicine to enhance sexual stamina, sensation and libidos. It is used to increase libido in both men and women due to its dopamine inducing properties (Amin et al, 1996).

Mucuna pruriens seeds have also been found to have antidepressant properties when consumed. It is a very beneficial supplement for body builders as it increases the body's ability to build lean muscle and breakdown fat, and is highly recommended to sportsmen (Ray Sahelian, M.D). An isolation made on the seed revealed that the seed of *Mucuna pruriens* (utilis) contains Estra – 2^{II} – en – 17 – ol, 3yl benzoate which suggested the reason why *Mucuna pruriens* seeds are used by the natives in the treatment of disorders of the male and female reproductive systems, parkinson disease and increase libido in both men and women and as anti- depressant (Uchegbu and Echeme, 2013). In spite of the numerous uses of *Mucuna pruriens* seeds in herbal medicine, compounds responsible for the biological and physiological activities have not been fully isolated and documented, thus this paper presents another isolation made from the seed of *Mucuna pruriens.*

2.0 Materials and Method:

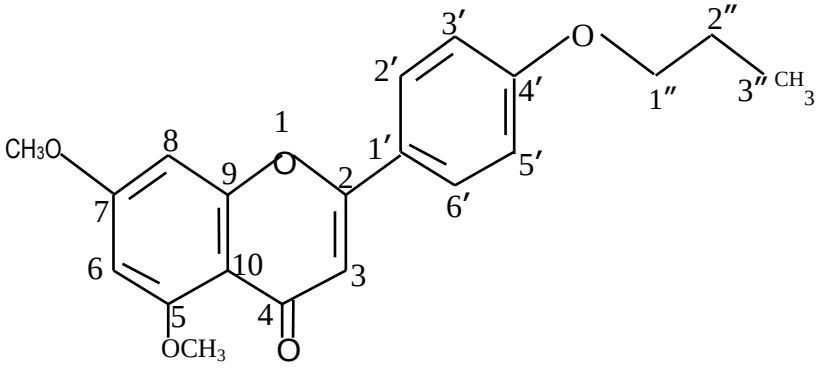
2.1 Plant Materials : The seeds of *M. pruriens* were harvested from the field of National Root Crops Research Institute Umudike ,Abia state, Nigeria. Authentication of plant materials was done by Dr A. Nmeregini of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.
2.2 Extraction and Isolation of Plant Materials : The seeds were cracked, the testa were removed and the seeds were milled into fine powder with Thomas Willey milling machine and then stored in air tight bottles for analysis. 2kg of the sample was percolated in 98% ethanol for 48hrs, this was then filtered. The filtrate was concentrated with Rotary evaporator at 40^{o}C to a dark brown crude extract (44.3g). The crude extract was

partitioned between $CHCl_3$ and water and a $CHCl_3$ - soluble fraction (17.5g) was obtained. 10.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60 – 80^0C) and aqueous methanol. 5.0g of the $CHCl_3$ fraction was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100ml petroleum ether, then petroleum ether : $CHCl_3$ (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90;), and 100ml $CHCl_3$; then $CHCl_3$: Methanol (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90) and 100ml methanol to yield ten major fractions labeled : G (yellow oil, 0.3g), I (yellow oil,0.4g), b (brown oil, 0.3g) c_1 (yellow oil,0.6g), sc_2(yellow oil, 0.60g),d (brown oil 0.32g) f_2 (brown solid, 0.3g), g_2 (brown oil, 0.5g), h (dark brown oil, 0.4g), i (light yellow oil, 0.3g) and i_2 (light yellow oil, 0.4g). Chromatographic (partition chromatography, column chromatography, and TLC) and spectroscopic (IR, ^{1}HNMR, ^{13}CNMR and MS) techniques were employed to isolate, characterize and identify active constituents from $CHCl_3$ extracts of the seeds.

3.0 Results and Discussions:

Compound [1] was isolated as yellow oil (0.7g) with $CHCl_3$ and Methanol in the ratio of 40:60. Based on combined analyses of HREIMS (m/z 339.30), ^{1}HNMR, ^{13}CNMR and IR spectra, compound [1] was proposed as 5, 7 – dimethoxy, 4' – propoxyflavone with molecular formula $C_{20}H_{20}O_5$ m/z 340 calculated. Analysis of IR is shown in Table 1 and the analysis of ^{1}HNMR and ^{13}CNMR are shown in Table 2. The IR spectrum showed Vmax 3007.63cm^{-1} (C=C), 2924.94cm^{-1}(-CH_2), 2855.71cm^{-1}(-CH_2), 1743.92cm^{-1}(C=O), 1166.22(C-O)

.



[1] 5, 7 – dimethoxy, 4′– propoxyflavone

Table 1: Infra-red Analysis of 5,7 – dimethoxy, 4'– propoxyflavone [1] from *Mucuna pruriens* Seed

IR ABSORPTION(CM^{-1})	FUNCTIONAL GROUP	COMPOUND TYPE
3007.63	C=C	Aromatic
2924.94	-CH_2	Aliphatic
2855.71	-CH_2	Aliphatic
1743.92	C=O	Carbonyl
1166.22	C-O	Ether

Table 2: ^{1}HNMR and ^{13}CNMR Chemical Shifts of Compound [1]

	δC		δH		
POSITION	**CHEMICAL SHIFT(δ)**	**CARBON**	**CHEMICAL SHIFT(δ)**	**MULTIPLICITY**	**PROTON**
1	_	_	_	_	_
2	77.36	C	_	_	_
3	128.04	C	5.283	1Hs	CH
4	173.42	C=O	_	_	_
5	65.17	C	_	_	_
6	128.18	C	5.308	1Hs	CH
7	69.02	C	_	_	_
8	128.20	C	5.312	1Hd	CH
9	77.16	C	_	_	_
10	129.84	C	_	_	_
11	53.55	OCH_3	2.740	3Hs	OCH_3
12	62.23	OCH_3	2.760	3Hs	OCH_3
1’	129.88	C	_	_	_
2’	130.11	C	5.331	1Hd	CH
3’	130.14	C	5.338	1Hd	CH
4’	77.58	C	_	_	_
5’	130.17	C	5.349	1Hd	CH
6’	130.35	C	5.358	1Hd	CH
1’’	76.74	CH_2	2.275	2Ht	CH_2
2’’	31.92	CH_2	2.006	2Hm	CH_2
3’’	29.26	CH_3	1.245	3Ht	CH_3

S = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

The ^{1}HNMR spectrum showed the presence of aromatic protons at δ 5.283 to δ 5.358, methylene protons at δH 2.275 (2Ht) and δH 2.006 (2Hm) and methyl protons at δ1.245 (3Ht). The ^{13}CNMR spectrum of compound [1] showed a carbonyl carbon at δC 173.42 and aromatic carbons at δ 128.04 - 130.35. The methylene carbons showed absorption at δC 76.74 and δC 31.92. The methyl proton showed its absorption at δC 29.26. The fragmentation pattern of compound [1] is showed in fig [1]. Detachment of the C_3H_7O fragment produced the peak at m/z 59. Another detachment of C_9H_5O and $C_8H_8O_3$ fragments from the flavones produced the peaks at m/z 129 and m/z 152 respectively.

Flavones are mainly found in cereals and herbs. In recent years, scientific and public interest in flavones have grown enormously due to their beneficial effects against atherosclerosis, osteoporosis, diabetes mellitus and certain cancers such as breast cancer. Many flavones have been isolated from plants and most of them have been reported to have antibacterial, antioxidant and anti-inflammatory activities (Veitch and Grayer, 2008). Thus the presence of this isolated compound in the plant may be the reason why *M. pruriens* is used in traditional medicine to treat elephantiasis, infections and to heal wounds.

Conclusion

The result of this analysis showed that *Mucuna pruriens* plant can serve as source of quality raw material for pharmaceutical industries for production of useful drugs. It therefore contributes to the scientific evidence for the use of this medicinal plant in herbal medicine for the treatment of oedema, infections, wounds e.t.c.

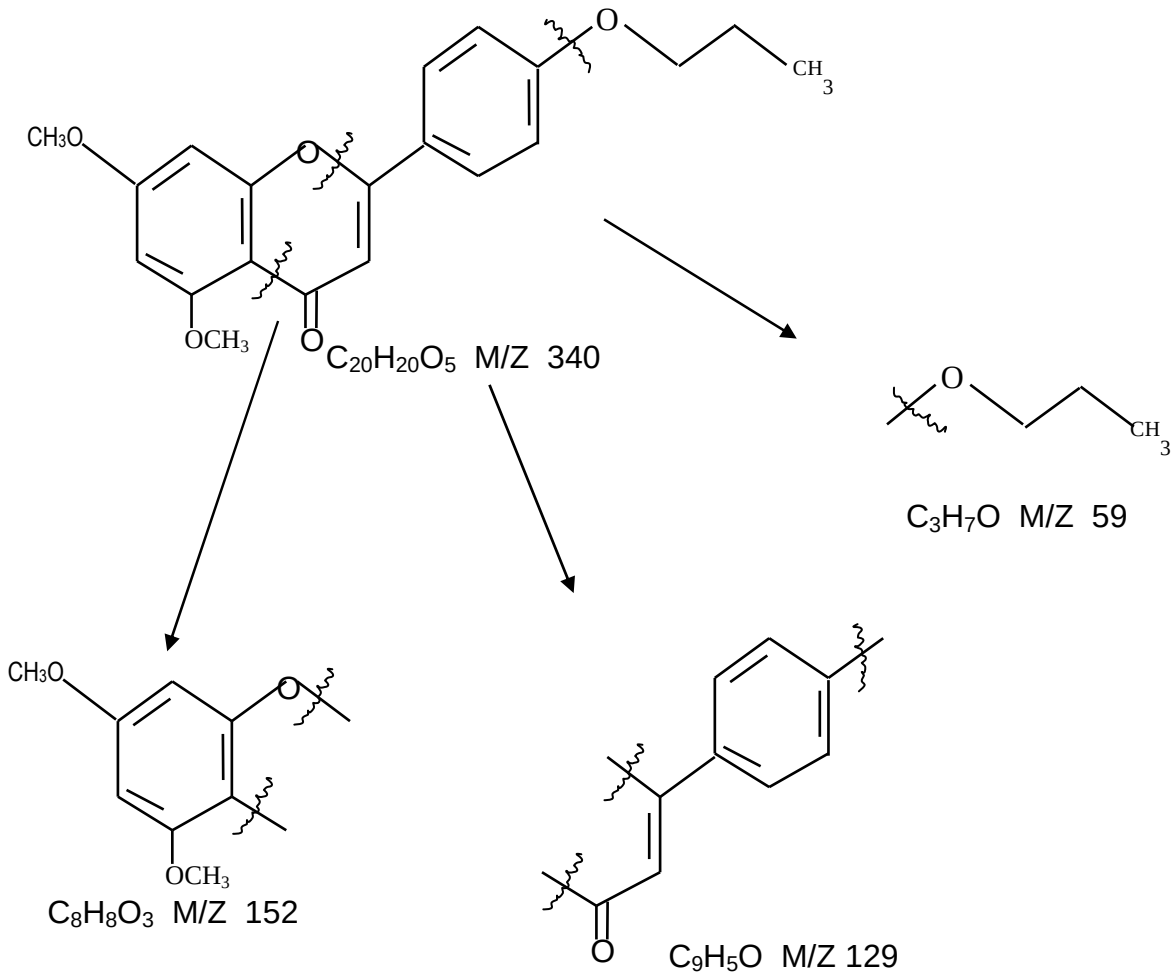


Fig 1: Fragmentation Pattern of Compound [1]

Acknowledgement

Authors are grateful to Dr Nkechi Ibisi of Chemistry department, Micheal Okpara University of Agriculture, Umudike, Nigeria for running our spectra in China and to Dr A. Nmeregini of Taxonomy section, Forestry department, Micheal Okpara University of Agriculture, Umudike, Nigeria for authenticating our sample.

References

Amin, K.M.Y, Khan, M.N and Zillur-Rehman S, (1996) "Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats".

Buckles D.,Triomphe, B and Sain,G (1998).Cover crops in Hillside Agriculture: Farmer Innovation with Mucuna.IDRC/CIMMYT.

Burkill, H.M (1995).The useful Plants of West Tropical Africa.(vol 3).Royal Botanic Gardens,London .Pp 101.

Duke J.A. (1981). Handbook of legumes of world economic importance. Plenum press. New York.

Ezueh, M.I (1997) Cultivation and Utilization of Minor Food Legumes in Nigeria. Tropical Grain legume Information Center, IITA, Ibadan, Nigeria .

Giuliano, F. and Allard, J.(2001). Dopamine and sexual function. *Int J Impot Res 13 Suppl* 3:18-28

Katzenschlager, R.; Evans, A and Manson, A (2004). Mucuna pruriens in Parkinson's disease: a double blind clinical and pharmacological study. *J Neurol Neurosurg Psychiatry* 75:1672-1677

Manyam,B.U.; Dhanasekaran,M. and Hare,T.A (2004).Neuroprotective effects of the antiparkinson drug Mucuna Pruriens. *Phytother Res* 18:97-101.

Numida, M.L (2009). "The effects of Raw and Processed *Mucuna pruriens* seed Based Diets on the growth parameters and meat characteristics of Benin Local Guinea Fowl". *International journal of Poultry sciences* 8(9).

Ray Sahelian, M.D. Accessed at www.raysahelian.com/mucunapruriens.html.

Udensi, E.A and Eke, O. (2000). Proximate Composition and Functional Properties of Flour Produced from *Mucuna cochinchinensis* and *Mucuna utilis*. Food and Fiber production in Nigeria in the 21st century. Pg 170 – 173.

Uchegbu, R.I and Echeme, J.O (2013). Isolation and Characterization of Estra – 2'' – en 17 – ol, 3yl benzoate from *Mucuna pruriens* (Utilis). *Journal of Natural Sciences Research* 3 (11). Pg 84 – 87.

Vadivel,V (2010). "Studies on the incorporation of velvet bean (*Mucuna pruriens var utilis*) as an alternative protein source in poultry feed and its effect on growth performance of broiler chickens.Tropical Animal Health and Production.

Veitch, N.C and Grayer, R.J. (2008). Flavonoids and their glycosides, including anthocyanins. *The Royal Society of Chemistry journal* (1): 557, 579,585.