International Journal of Engineering and Technology Studies
Vol.1, No.1, pp.21-30, March 2014
Published by British Research Institute UK (www.gbjournals.org)

ASSESSMENT OF MUCUNA PRURIENS (COW-ITCH) AS A POTENTIAL BIOLOGICAL WEAPON

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ABSTRACT: *The potential of Mucuna pruriens as a biological weapon (itch - inducer) was assessed by percolating the dry hairs of the plant and subsequent column chromatographic separation of the extract obtained using n- hexane, ethyl acetate and ethanol as eluents. Human volunteers were used for the timing of itch duration at various concentrations. The n-Hexane extract did not itch any of the subjects, ethyl acetate extract itched averagely for 38secs at 0.02g/cm^3, 76.6secs at 0.04 g/cm^3 and 118secs at 0.06g/cm3. Pure ethanol extract itched for 51.2secs at 0.02g/cm3, 101.4secs at 0.04g/cm^3 and 151.8secs at 0.06g/cm^3. Methylation of the ethanol extract which had the highest itching potency and the dry Mucuna pruriens hairs with sodium methoxide increased the itching potency of Mucuna pruriens which was noticed in the increased duration of the itch: 53.4secs, 107.6secs and 160.6secs for methylated ethanol extract; 56.6secs, 112.6secs and 169.4secs for methylated dry Mucuna pruriens hairs in order of increasing concentration of the extracts: 0.02, 0.04, 0.06g/cm^3 respectively. The results of GC-MS revealed a series of fatty acids with Oleic acid having the highest percentage (79.6%) in all the extracts analysed. Methylation using sodium methoxide converted most of the oleic acid into a methyl ester (methyl-14-methylpentadecanoate). Other fatty acids detected include, stearic acid, palmitic acid, erucic acid and myristic acid. The functional groups of the compounds responsible for itching in Mucuna pruriens were confirmed to be present in the IR results. Based on the results, methylated ethanol extract and methylated Mucuna pruriens hairs could serve as good sources of biological weapon.*

KEYWORDS: Mucuna pruriens, Itch, Methylation, Serotonin, Mucunain, Biological weapon.

INTRODUCTION

Mucuna pruriens commonly called cowitch or cowhage in English, 'Karara' in Hausa, Yerepe in Yoruba and 'Agbala- ugwu in Igbo is an annual habaceous twining legume which belongs to the family Fabaceae (Leguminosae). It is indigenous to tropical regions especially Africa, India and West Indies (Rajandran *et al*., 1997). The plant possesses white to dark purple flowers and its fruits are enclosed in a pod, which is thick and hairy. The seed is characterized by the presence of a single layered palisade and vascular sac containing large oval shaped grains (Akhatar, 1990; Buckles *et al*., 1998). The leaves and seeds of *M. pruriens* are a good source of protein for ruminant animals. Studies have shown that it is used occasionally as minor crop for human consumption (roasted beans as coffee substitute, cooked beans and young leaves as vegetables) (Carsky *et al*., 1998; Eilittä *et al*., 2003). Dried leaves of *M. pruriens* have stimulating effect (Eilittä *et al*., 2003). The herb has been reported

to contain Levo- Dopamine commonly known as L- Dopa. It is a precursor to the neurotransmitter dopamine [(2, 4-dihydroxyphenethylamine ($C_{17}H_{34}O_2$)] which increases when extracts are prepared and converts into dopamine (Eilittä *et al*., 2003). The hairs lining the pods and the spicules on the leaves of *M. pruriens* (cow itch), usually the wild specie contain a cysteine protease called *mucunain* and 5-hydroxytryptamine also known as serotonin which together elicit pruritus (severe itching) when in contact with human skin. (Yerra *et al*., 2005).

The conventional chemical sprays- Oleoresin capsicum OC, Orthochlorobenzalmalononitrile, CS and Chloroacetophenone, CN used over the years in conflict control or during an uprising have reported lethal effects when used in high concentrations. The use of methylated hairs of *Mucuna pruriens* compressed in the form of a micropulvirised spray as explored in this research may serve to expose enemies from their hiding places due to the strong itching effect elicited by the hairs of the plant. This compliments the conventional chemical sprays and also provides a cheaper and equally effective alternative form of biological weapon to be used for offence or defence.

MATERIALS AND METHOD

Sample Collection (Plant Source)

The mature pods were harvested from the plant's natural habitat in February and March, 2012 (dry season) at Dan Bushiya- Millenium City, Chikun Local Government area of Kaduna State. Identification of the plant was done at the Department of Biological Sciences by Mallam Musa Galla of the herbarium section of Ahmadu Bello University Zaria, Kaduna state, Nigeria (Voucher Number 900231).

Sample Preparation

This was done by scraping the *Mucuna pruriens* hairs off the pods of the plant using a clean spatula into a conical flask of 500cm^3 capacity. The spatula and conical flasks used were washed with soapy water, rinsed and properly dried in the oven prior to use.

Extraction of Plant Material

5g of *M. pruriens hairs* was percolated in 250cm^3 of ethanol at room temperature for 21 days and then filtered. The filtrate was evaporated using Rotary evaporator at 40^{o}C to obtain the crude, brownish solid extract which was weighed and kept in the refrigerator until needed for further analysis.

Column Chromatography

1g of the crude *M. pruriens* extract was mixed with a filter agent (celite) and silica gel (1g). The mixture was loaded on a silica gel column (27g silica gel; I.D 1.2cm) and eluted with n-Hexane (200cm^3), ethyl acetate (200cm^3) and ethanol (200cm^3). Each fraction was evaporated to dryness using Rotary Evaporator at 40^{0}C, and stored in labeled beakers in preparation for further analysis.

Potency test of column chromatographed fractions

Skin test was performed on the three extracts to find out which fraction had the highest potency. This was based on the intensity of the itching sensation elicited by each fraction and the duration of the itch. A total of 5 human volunteers participated in the study. All the volunteers were ascertained to be free of allergies, atopic eczema or other dermatological diseases by enquiry about details of their medical history. 0.2g of each solid fraction was dissolved in $2cm^3$distilled water to obtain an approximate solution of $0.1g/cm^3$. Using a micro pipette, $0.4cm^3$, $0.6cm^3$ and $0.8cm^3$ of the stock were transferred into $2cm^3$ veils and made up to the mark with distilled water to a concentration of 0.04g/cm3, 0.06g/cm3 and 0.08g/cm3 respectively. 2 drops of these solutions were applied to the epidermal layers of all the subjects. Using a stop watch, their respective responses were recorded over a time frame through which the itch lasted.

Preparation of Sodium Methoxide

Sodium methoxide was prepared by carefully treating methanol with sodium metal:

$2Na_{(s)} + 2CH_3OH_{(aq)} \rightarrow 2CH_3ONa_{(aq)} + H_{2(g)}$ (El-Kattan, *et al*., 2006).

11.5g of sodium metal was placed in a flask which was attached to a reflux condenser and immersed in cold water bath. 115 cm^3 of methanol was slowly added through the condenser. When the initial vigorous reaction moderated the cold water bath was removed. This preparation was concluded within 40 minutes. (Mike, P., 2001; Tishler in Fieser, 1941; Allen *et al*., 1928)

Methylation

Methylation of the ethanol extract and dry *M. pruriens* hairs with sodium methoxide was done by refluxing $30cm^3$of ethanol extract with $30cm^3$ of the prepared sodium methoxide for 2 hrs. The same procedure was repeated using 0.5g of the dry *M. pruriens hairs* in $30cm^3$ of sodium methoxide. Both methylated products were stored in two separate labeled sample bottles for potency test.

Potency test of methylated extracts

Skin test was carried out on five (5) human volunteers to assess the itching power of the methylated ethanol extract and also the crude, methylated dry hairs of *M. pruriens*. The assessment was based on the intensity of itch elicited by each of the specimens listed as a function of time as felt by the subjects. 0.2g of each solid fraction was dissolved in $2cm^3$ distilled water to obtain an approximate solution of $0.1g/cm^3$. Using a micro pipette, $0.2cm^3$, $0.4cm^3$ and $0.6cm^3$ of the stock were transferred into $2cm^3$ veils and made up to the mark with distilled water to a concentration of $0.02g/cm^3$, $0.04g/cm^3$ and $0.06g/cm^3$ respectively. 2 drops of these solutions were applied to the epidermal layers of all the subjects. The respective responses of the subjects over time were recorded using a stop watch.

RESULTS AND DISCUSSION

The results of potency tests of the three different extracts obtained by percolation and subsequent column chromatographic separation, methylated ethanol extract and methylated crude dry *M. pruriens* hairs are shown in Tables 1-5. Tables 6-8 show the results of GC-MS analysis of pure ethanol extracts, methylated ethanol extract and methylated dry *Mucuna pruriens* hairs. The GC- MS results as represented were further analyzed using chem office software to obtain the compounds which had the highest percentage. Table 9 shows the IR results for pure ethanol extract, methylated ethanol extract and methylated dry *Mucuna pruriens* hairs.

Table 1: Result of potency test for n- Hexane extract

Number of volunteers	Response	Duration of Itch
X1	No Itching	0 seconds
X2	No Itching	0 seconds
X3	No Itching	0 seconds
X4	No Itching	0 seconds
X5	No Itching	0 seconds

Table 2: Result of potency test for ethyl acetate extract

Concentration (g/cm^3)	**Volunteers**	**Duration of itching (Seconds)**	**General response**
0.02	X_1	30	Mild itching
	X_2	35	
	X_3	50	
	X_4	27	
	X_5	48	
0.04	X_1	62	Mild itching
	X_2	72	
	X_3	101	
	X_4	53	
	X_5	95	
0.06	X_1	91	Mild itching
	X_2	106	
	X_3	153	
	X_4	90	
	X_5	150	

Table 3: Result of potency test for pure ethanol extract

Concentration (g/cm^3)	Volunteers	Duration of itching (Seconds)	General response
0.02	X_1	44	Mild itching
	X_2	50	
	X_3	60	
	X_4	44	
	X_5	58	
0.04	X_1	87	Strong itching
	X_2	98	
	X_3	121	
	X_4	86	
	X_5	115	
0.06	X_1	131	Strong itching
	X_2	148	
	X_3	179	
	X_4	129	
	X_5	172	

Table 4: Result of potency test for methylated ethanol extract

Concentration (g/cm^3)	Volunteers	Duration of itching (Seconds)	General response
0.02	**X_1**	**47**	**Strong itching**
	X_2	**55**	
	X_3	**60**	
	X_4	**47**	
	X_5	**58**	
0.04	**X_1**	**95**	**Strong itching**
	X_2	**111**	
	X_3	**122**	
	X_4	**92**	
	X_5	**118**	
0.06	**X_1**	**140**	**Severe itching**
	X_2	**166**	
	X_3	**182**	
	X_4	**139**	
	X_5	**176**	

Table 5: Result of potency test for Methylated *M. pruriens* hairs (spicules)

Concentration (g/cm^3)	Volunteers	Duration of itching (Seconds)	General response
0.02	X_1	50	Strong itching
	X_2	57	
	X_3	65	
	X_4	48	
	X_5	63	
0.04	X_1	101	Severe itching
	X_2	113	
	X_3	127	
	X_4	98	
	X_5	124	
0.06	X_1	149	Severe itching
	X_2	170	
	X_3	196	
	X_4	144	
	X_5	188	

Key: X1- X5 are the volunteers.

Table 6: GC-MS result for pure ethanol extract of *Mucuna pruriens* hairs

S/ No.	Constituents	Percentage (%)	Retention time	Retention index
1	Oleic acid	29.9	24.842	2175
2	9-octadecenoic acid	20.8	24.225	2085
3	Hexadecanoic acid	17.2	21.717	1878
4	Tetradecanoic acid	16.7	22.708	1769
5	Tetradecanol	10.8	27.358	1609
6	14-methyl-8-hexadecanal	2.6	29.600	1843
7	1,2-benzenedicarboxylic acid	2.2	28.029	1834

Table 7: GC- MS results for methylated ethanol extract of *Mucuna pruriens* hairs

S/No	Constituents	Percentage (%)	Retention time	Retention index
1	14-Methyl-14-pentadecanoate	27.8	24.258	1814
2	Octadecanoic acid	24.2	24.258	2085
3	Hexadecanoic acid	17.4	21.742	1878
4	Butyl-1-octanol	13.3	25.942	1393
5	13-octadecedien-1-ol	12.3	27.367	1843
6	Hexadecanal	3.4	24.808	1808
7	2-hydroxy-1,3-propanedilester	1.6	25.942	4395

Table 8: GC- MS results for methylated dry *Mucuna pruriens* hairs

S/No	**Constituents**	**Percentage (%)**	**Retention time**	**Retention index**
1	Oleic acid	27.3	24.247	2085
2	Metholene	23.9	21.733	1878
3	Hexadecanal	17.3	-	-
4	Octadecanoic acid	15.4	24.458	2077
5	Erucic acid	8.0	24.800	2572
6	Sterric acid	7.9	25.942	4395

Table 9: Interpretation of IR spectra of Methylated *M. pruriens* hairs (MMH), Methylated ethanol extract of *M. pruriens hairs* (MEE) and Pure ethanol extract of *M. pruriens hairs* (PEE) Samples.

Sample	Absorption frequency (cm^{-1})	Type of bond	Type of compound
MMH (Methylated Mucuna hairs)	3726.60	N-H	Amines
	3580.00	N-H	Amines
	3344.66	OH	Carboxylic acids/phenols
	2918.40	C-H	Alkanes
	1791.93	C=O	Carboxylic acids
	1325.14	C-O	Ester/ carboxylic acids
	837.13	C-H	Aromatic ring C-H
MEE (Methylated ethanol extract)	3772.89	N-H	Amines
	3392.90	C-H	Carboxylic acids
	3192.30	C-H	Aromatic ring C-H
	2671.50	C-H	Aldehydes
	1606.76	C=O	Finger print for esters
	1300.07	C-O	Carboxylic acids/esters
PEE (Pure ethanol extract)	3749.94	N-H	Amines
	3553.00	N-H	Amines
	3190.32	O-H	Carboxylic acids
	2744.80	O-H	Alcohols/phenols
	2264.51	CN	Nitriles
	1766.85	C=O	Carboxylic acids
	1450.52	C-H	Alkanes

DISCUSSION

The results of this research showed that n- hexane extract did not elicit itch in all the subjects as represented in table 1. This may be due to the fact that *mucunain* and serotonin being the compounds responsible for the itching sensation felt on contact with the hairs of *M. pruriens* as reported by Reddy and Lerner et al in 2010 are not soluble in n- hexane. Table 2 shows that mild itching was reported for ethyl acetate extract which lasted averagely for a few seconds. This indicates that serotonin and mucunain may probably be slightly soluble in ethyl acetate. The variation observed in the duration of the itching as felt by the subjects indicates difference in central nervous system processing of the released compounds in the skin due to individual differences in suppressing the itch- processing as earlier reported by Frauke *et al.* in 2009.

The strongest itching sensation was obtained from ethanol extract because mucunain and serotonin are polar in nature and are therefore soluble in ethanol (See Table 3). This variation observed in the duration of the itch supports the findings of Frauke *et al*., 2009 and Yosipovitch *et al*., 2007 which revealed that response to itch varies individually due to difference in nervous processing and suppression of itch.

Methylation of the ethanolic *M. pruriens hairs* extract and dry *M. pruriens* hairs increased the itching felt by the subjects. The methylated plant's ethanol extract initiated a strong itch faster than the methylated dry *M. pruriens* hairs. The methylated *Mucuna* hairs however had stronger itching which lasted longer than the itching caused by the methylated ethanolic extract. Considering the methylated ethanolic extract (see Tables, 5 and 6). A notable variation was observed in the duration of itch as the concentration of the extracts was generally increased in all the subjects for the respective extracts analyzed. The trend observed is in agreement with the findings of Frauke *et al*., 2009 and Yosipovitch *et al*., 2007 on individual differences regarding itch processing. Methylation of the ethanol extract of *M. pruriens hair* and dry *M. pruriens* hairs both increased the itching felt by the subjects as shown in Tables 7 and 8. This may be due to the burning sensation which naturally accompanies pure sodium methoxide.

The results of GC- MS showed series of compounds which were mostly fatty acids (carboxylic acids). In the pure ethanol extract, Oleic acid (9- octadecenoic acid) had the highest percentage (50.7%) followed by hexadecanoic (palmitic)acid (17.2 %), 16.7% tetradecanoic (myristic) acid and tetradecanol (myristyl alcohol) (10.8%). Other compounds detected in the pure ethanol extract are 1, 2-benzenedicarboxylic acid also called phthalic acid (2.2%) and 14- methyl-8-hexadecanal (2.6%), (see Table 6).

The compounds to which the itching caused by *Mucuna* hairs is attributed (mucunain and serotonin) as reported by Reddy and Lerner in 2010 were not clearly detected by the GC- MS analysis of the samples. However, the presence of these compounds was evident in the IR results (see Table 9)

CONCLUSION

Based on findings of the research, the quality of the itch already resident by nature in the hairs of this plant was increased by methylation with sodium methoxide which increased the burning sensation that naturally accompanies contact with the hairs of the plant. Therefore the results showed that the chemical compounds responsible for itching in *Mucuna pruriens* are not soluble in n- hexane, only slightly soluble in ethyl acetate and highly soluble in ethanol. Based on the results, methylated ethanol extract and methylated *Mucuna pruriens* hairs could serve as good sources of biological weapon.

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