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Pharmaceutical Constituents of Seed and Seedcoat of *MucunaPruriens*(Velvet Bean)

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ABSTRACT

This work studied the pharmaceutical constituents of seed and seedcoat of Mucunapruriens. Qualitative and quantitative phytochemical Screening of seed and seedcoat of Mucunapruriens showed the presence of these phytocompounds: alkaloid = 39.21± 0.014% tannins = 19.07± 0.001%, Saponic glycosides = 46.03± 0.014%, Flavonoid = 17.04± 0.01%, Phenolic glycoside = 17.04± 0.01%, terpenoids =2.43± 0.00%, antrachionomic glycoside = 4.09± 0.0141%, cardiac glycoside = 7.70± 0.014%. The ethanolic , n – hexane and aqueous extracts of the seeds and coat of Mucunapruriens were found to inhibit two test gram positive bacteria, two test gram *negative bacteria and two test fungi. The two gram positive bacteria are; S. aureus and B. subtilis. The two gram negative bacteria are: E. coli and S. pyogene. The two fungi are: C. albican and A. flavus. The minimum inhibitory concentration for the three solvent extracts were found for each bacteria and fungi. Elemental analysis showed that the seeds of Mucunapruriens contain the following microconstituent elements: Ca, Mg, P, Na, and K.*

Introduction

Mucunapruriens is commonly known as velvet bean in Nigeria, Australia, South Africa and USA, Pica Pica in Venezuela and Bengal bean in India (Skerman et al,1977) . *Mucuna* is an animal twining plant (Internet 1, 2011). It is a native of southern Asia, Malaysia and widely distributed in the tropics. Velvet bean is universally used in Nigeria as a soil improvement crop/cover crop and can trail on trees (Webster,1938). *StizolobiumSpp (synMucunapruriens)* is a vigorously growing trailing vine, slender, slightly ridged and extending over 6m in lengths (they may grows to over 10m). The leaves are large and smooth, the terminal leaf being rohomboidatovate and the lateral once oblique, 20 to 25 cm long and 7.5 to 12.5cm wide. The flowers are born in long racemes (15-30cm, long hanging clusters) white with purpose tinge. The pod (10 to 14 in a cluster) are born singly, 10-12.5cm long, curved (S-shaped) with a grayish-white pubescence of short silky hairs.

Some varieties have irritating, itching powdery fuzz on the pods with mottled seeds. The pod is green when young and black when dried. The seeds are black large, glossy and sub-globose when mature (Duke, 1981), 1.2 to 1.5 cm long and 0.9 to 1.1cm broad with raised white helium half as long as the seed. Each pod contains 3 to 5 seeds (Paul, 1951). *Mucunapruriens* is a wide spread fodder plant in the tropics. The whole plant is fed to animals as silage, dried hay or dried seeds. The seed of mature, unriped or young pods of *M. pruriens* are soaked in water and boiled or /roasted and eaten as such or mixed with salt and eaten by the poor North-east India tribes (Arora, 1981). Some Indian tribes consume the seeds for increased potency and the hairs of the pods are used as vermifuge (VasudevaRao et al,1981). *Mucunapruriens* bean is consumed in several parts of Sri-lanka, particularly by the low-income group and it is also used in indigenous 'ayurveaic' medicine (Jayaweera,1981) .

M. pruriens seeds contain high level of L. dopa. This requires that they be soaked from at least 30min to 48hours in advance of cooking or the water used in cooking be changed up to several times during cooking. This process leeches out chemical and compounds such as L. dopa, making the seeds suitable for consumption. The acceptability and utilization of legumes as human food or animal feed has been limited due to the presence of relatively high concentrations of certain anti-nutritional factors such as lectins, protease inhibitors, and x-amylase inhibitor, allergens, polyphenols and phytic acid (Liener,1994). *M. pruriens* from India has been reported to contain trypsin inhibitors, phytates, cyanogenic glycosides, tannins, phenols, α-amylase inhibitor and L-3-4 dihydroxphenylalanine (L-DOPA) (Ravindran et al,1988 ; Josephine et al,1992). Siddhurajuetal reported the presence of lectins in the seed of *M. pruriens (Siddhuraju et al,1996* . These anti-nutritive factors are found to be drastically reduced by dry heat and autoclaved methods (Onwueyiagba,2001).

Experimental

Sample collection and preparation:

The fresh seeds of *M. pruriens*were obtained from Umuabu village, Adazi-Enu in Anaocha L.G.A. of Anambra State, Nigeria. The seeds were washed, dried under the sun for two weeks and ground into powder. It was then stored in polyethylene bag until needed for analysis.

Physio-chemical, phytochemical analysis and extraction of the active components were determined by the methods outlined by (Harbon, 1973). The antimicrobial activity of seeds and seedcoat of *M. pruriens*was determined by agar well diffusion method (Okeke et al, 2001).

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Table 1. Result of physio- chemical characteristics of seed extracted drugs of *M. pruriens* in three different solvents: Ethanol, N-hexane and water.

Parameter	Ethanol	N-hexane	Water
Appearance and Texture	Dried and semi crystallized	Dried and sticky	Dried and powdery
Colour	Dirty brown	Brown	Yellowish brown
Odour	Slightly chocking	Characteristics	Characteristics
Solubility	Moderate soluble	Partially insoluble	Soluble
Taste	Sour and astringent	Sour and astringent	Bitter and astringent
pH at 25^0C (10%)	7.3	7.1	7.4
Percentage yield of Drug extract	3.88	3.01	4.80

Table 2. Phytochemical screening of seeds of *Mucunapruriens*

*C*onstituents	Bioassay
Alkaloids	+
Tannins	+
Saponic Glycosides	+
Flavonoids	+
Phenolic Glycosides	+
Terpenoids (essential oils)	+
Anthracinic Glycoside	+
Cardiac Glycoside	+

+ means present.

The zone of inhibition was recorded to the nearest size in mm (Norrel et al, 1999). Graphical methods was used in determination of minimum inhibitory concentration (MIC) of dried seeds and seedcoat of *M. pruriens*

After extraction of the active components using three different solvents (Ethanol,N-hexane and Water), the extracts were evaporated to dryness at about 40^oC in a water bath separately. 0.1, 0.2, 0.3, 0.4 and 0.5g of dried ethanolic, n-hexane and water extracts were weighed into five different labeled test-tubes differently. Then 1ml of the corresponding solvent used for extracting was added to the dried extract. That is ethanol for ethanolic extract in five test-tubes, n-hexane for n-hexane extract in another five test-tubes and water for water extract in another different five test-tubes.

Results

The results of all the analysis carried out on seeds of ***M. pruriens*** for its active constituents present are given in tables 1-6.

Table 3. Quantitative estimates of phytochemical constituents of seeds of *Mucunapruriens*.

Constituents	Quantitative %
Moisture contents	9. 55±0.014
Alkaloids	39.21± 0.013
Tannins	19.07±0.001
Saponic Glycosides	46. 03±0.014
Flavonoids	17. 04± 0.012
Phenolic Glycosides	17. 04± 0.010
Terpenoids (essential oils	2.43± 0.021
Antrachionic Glycoside	4.09± 0.0125
Cardiac Glycoside	7. 70± 0.011

Results are mean of triplicate determinations

Table 4. mineral Composition of the Seeds of *M. pruriens*

Mineral	% concentration
Ca	0.22
Mg	3.08
P	1.05
Na	2.33
K	2.14

Discussion

From table 1, the pH at 10% of the extracts ranges from 7.1 to 7.4. This showed that the extracts could be suitable to be used as herbal drug since they are neither acidic nor too basic in nature. The crystalline, sticky and powdery nature of the various sample extracts suggested that the various extracts could be incorporated in pharmaceutical drugs in form of tablets, or capsules. The percentage drug yield, by the three extracts were appreciable ranging from 3.01 to 4.80, though that of the water extract was highest (4. 80).

Table 2 and 3 portrays both qualitative and quantitative estimates of phytocompounds present in seeds of *M. pruriens*. The phytochemical constituents of *M. pruriens* are as follows: alkaloids = 39.21± 0.013%, tannins = 19.07± 0.001%, saponic glycosides = 46.03± 0.014%, flavonoids = 17.04± 0.012%, Phenolic glycosides = 17.04± 0.010%, terpenoids = 2.43± 0.021%, antrachionic glycoside = 7.70± 0.011%. The alkaloids content of the *M. pruriens* seeds is appreciable. It could be used to remedy some diseases, depending on the type of alkaloids it contains. Plants rich in alkaloids were observed to possess the following properties; antisplasmodic, sedative, narcotic, antimitotic, antiviral, febrifuge, antimalarial, invigorating, wound healing and stimulating of gall bladder function. *M. pruriens* had analgesic and anti-inflammatory properties due to its alkaloid content (Evans,1996).

High percentage of saponin in the seeds of *M. pruriens*showed that it could be used to remedy diseases like constipation and some bacterial infections. *M. pruriens* seeds contain appreciable quantity of flavonoids and tannins. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anticancer activity (Salah et al,1995).

Table 5. Result of antimicrobial activity of crude extracts of seeds of *M. pruriens*on 2 Gram positive bacteria,2 Gram negative bacteria and 2 fungi

Test organism LBI	Concentration g/ml	Average diameter of zones of inhibition in MM on test organisms				
		Ethanolic extract	N-hexane extract	Aqueous extract	+ve control Antibiotic	-ve control 50% pure ethanol
Staphylococcus aureus	0.1	NA	NA	3.5	5.0	NA
	0.2	4.0	NA	7.5	10.8	NA
	0.3	10.0	2.0	1.2	16.05	NA
	0.4	15.0	6.0	19	22.3	NA
	0.5	21.0	9.0	18	28.1	NA
B. subtilis	0.1	NA	NA	NA	NA	NA
	0.2	3.0	NA	3.0	8.6	NA
	0.3	7.0	4.0	9.0	14.0	NA
	0.4	13.0	9.5	16.0	19.1	NA
	0.5	19.0	14.0	22.0	24.0	NA
Escherichia coli	0.1	NA	5.0	2.0	4.7	NA
	0.2	3.0	10.5	8.0	11.0	NA
	0.3	9.0	15.5	16.0	16.8	NA
	0.4	17.0	22.0	21.0	22.4	NA
	0.5	22.0	27.0	28.0	27.9	NA
S. pyogene	0.1	NA	NA	3.0	3.4	NA
	0.2	NA	NA	7.0	7.0	NA
	0.3	0.6	3.0	15.0	11.1	NA
	0.4	3.0	7.0	20.0	17.1	NA
	0.5	5.0	12.0	25.0	21.3	NA
Candida albican	0.1	NA	0.5	2.0	1.6	NA
	0.2	1	2.0	4.0	3.6	NA
	0.3	3	4.5	8.0	9.0	NA
	0.4	4.7	7.0	12.0	16.0	NA
	0.5	6.0	11.0	18.0	23.0	NA
A. Flavus	0.1	0.5	NA	1.0	1.0	NA
	0.2	2.3	NA	3.0	3.2	NA
	0.3	4.0	NA	5.0	7.0	NA
	0.4	5.8	NA	6.5	11.1	NA
	0.5	7.2	NA	7.9	16.98	NA

Key NA = No action
LBI = Local bacterial isolate

The presence of tannin in the leaves extracts of *M. pruriens* supports its strong use for healing of wounds, varicose ulcers, snake bite and burns in herbal medicine (Igboko, 1983). The presence of phenols signifies that it could be used as antiseptics and anti-inflammatory drug on urinary system. Appreciable quantity of cardiac glycoside in the seeds indicates that it could be used to increase the contractile strength of the heart and to regulate heart beat rhythm.

Elemental analysis in Table 4 showed that seeds of *M. pruriens* are good sources of these micro-constituent elements; Ca, Mg, P, Na, and K. Tables 5 depicts the result of anti-microbial activity of crude extracts of seeds of *M. pruriens* on:

1. Two Gram positive bacteria eg*S. aureus and B. subtilis*.
2. Two Gram negative bacteria eg*E. coli and S. pyogene*.
3. Two test fungi e.g. *C. albican and A. flavus* .

Five different concentration of the extracts were used. Aqueous extract of *M. pruriens* seed significantly inhibited the growth of all the test organisms-*S. aureus* 18mm, *B. subtilis* 22mm, *E. coli* 28mm, *S. pyogens* 25.5mm, *C. albican*15.4mm and *A flavus* 7.9mm. The water extracts was most effective against *E. Coli* with inhibition zone of 28.0mm. This was followed by S. pyogens 25mm, *B subtilis* 22mm, *S. aureus* 18mm, *C. albican* 18 mm and *A. flavus* 7.9mm. The extracts exhibited anti-bacteria activity due to the presence of tannins, saponins, flavonoids and alkaloids (Rabe et al,1987). At 0.5g/ml concentration of the extract, ethanolic extract inhibited all the test organisms, but at 0.1g/ml concentration, it cannot inhibit all the test organisms with the exception of one test fungus-*A flavus* with zone size of 0.5mm.

At 0.5g/ml concentration of the extract, ethanolic extract of *M. pruriens* seeds, showed highest efficacy against *E. coli* with inhibition zone size of 22mm. This was followed by *S. aureus, B. subtilis A. Flavus, C. albican and S. pyogenes* with zone sizes of 21.0mm, 19.0mm, 7.2mm, 6.0mm and 5.0mm respectively. From 0.1-0.5g/ml concentration, N-hexane extract cannot inhibit the growth of one of the two test fungi-*A. flavus*. This is because the N-hexane extracts has no effect on the fungus. At 0.1-0.2g/ml concentration, N-hexane extracts can only inhibit two of the six test organisms. These are*E.coli and C. albican.* At 0.5g/ml concentration, N-hexane extracts of *M. pruriens* seeds was most effective against *E. coli.* Positive control against the six test organisms using standard antibiotic, inhibited their growth but the negative control using 50% pure ethanol showed no effect on the growth of the six test organisms.

Table 6 symbolizes the result of the minimum inhibitory concentration (MIC) of three solvent extracts of *M. pruriens*seeds on six test organisms. The MIC of ethanolic extracts against the six test organisms ranges from 0.5g/ml to 0.25g/ml. The highest MIC of ethanolic extracts was shown against *S. pyogenes* (0.25g/ml) while the least was against *A.*

Flavus (0.05g/ml). The MIC of N-hexane extracts against the six test organism ranges from 0.01g/ml to 0.23g/ml. The highest MIC of N-hexane extract was shown against *S. pyogene* while the least was against *E. coli* (0.01g/ ml). N-hexane extracts has no effect on the fungus-*A.flavus*. The MIC of aqueous extract ranges from 0.02g/ml to 0.16g/ml. The highest MIC of aqueous extract was shown against *B. subtilis* (0.16g/ml) while the least was against *S. aureus* (0.02g/ml)

Table 6. Result of the minimum inhibitory concentration (MIC) of three solvent extracts of*M. pruriens*seeds on six test organisms.

Test organisms	Ethanol	N-hexane	Water
S. aureus	0.13	0.14	0.02
B. subtilis	0.8	0.22	0.16
E. Coli	0.16	0.01	0.07
S. Pyogene	0.25	0.23	0.05
C. albican	0.14	0.08	0.04
A. Flavus	0.05	NA	0.03

Conclusion and Recommendation

M. pruriens seeds contain many phytocompounds which are antimicrobial in nature. If *M. pruriens* seeds are to be used properly as pharmaceutical substances their anti-nutritive factors must be reduced to the barest minimum. This is done by soaking the seeds or by heating or autoclaving the seeds. *M. pruriens* seeds extracts can be used effectively in inhibiting the growth of the two tests:

1. Gram positive bacteria
2. Gram negative bacteria and
3. Fungi

These microorganisms are: *S. aureus, B.subtilis, E. Coli, S. pyogene, C. albican and A. Flavus.* In order words, *M. pruriens* seed extracts can be used to cure the diseases caused by the above enumerated micro-organisms. Generally, aqueous extracts have the lowest MIC. This means that the aqueous extracts of *M. pruriens* seed contain the smallest concentration of the crude drug that can inhibit the particular micro-organism in question. The use of *M. pruriens* seeds extracts should be encouraged in pharmaceutical and herbal medicine. It is therefore recommended that *M. pruriens* seeds extracts should be studied on more gram positive and gram negative bacteria. The investigation should also be extended on more fungi and virus.

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