PROXIMATE ANALYSIS OF VELVET BEANS SEED MUCUNA PRURIENS

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CERTIFICATION

This is to certify that this project research was written by **DEMBO HAWAU (HND/23/SLT/FT/778)** and submitted to the Department of Science Laboratory Technology, Biochemistry Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin and has been read and approved as a partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

This research work is dedicated to knower of all things, my Creator, Allah, the Lord of all the Worlds and its containment.

ACKNOWLEDGEMENT

To God Almighty belongs all the glory at the beginning and in the end. It is He who has made this research work possible for many and profound gratitude unto Him for keeping me sane and healthy all through school. May His peace and blessings be upon the noblest of mankind.

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To every person that has helped me in the course of this research, from friends to families, thank you so much. ll. Also, to everyone who have assisted or contributed in one way or the other to this 5years journey, I sincerely appreciate your beautiful contributions. To all and sundry, I pray Allah rewards us all abundantly**!**

ABSTRACT

Velvet beans (mucuna. P) seeds are conventionally used as food and medicine in the western Ghats of India. And its established herbal drug used for the management of male infertility, nervous disorder and also in an aphrodisiac. The present study aimed to analyse the proximate and mineral composition of India legumes seed. (while and black seed of velvet seeds) flour used by triba's of South India. The crude protein content was higher in black seed compared to white seed, but the nutritive valves of white seed were better compared to black seeds. The mineral constituents of both seed was found to be high in K, Ca, P and Na. The proximate and mineral element suggest that both seed varieties to be a cheap source of protein and macro element, therefore are useful food supplement. This wild legumes constitute not only a rich source of protein, but also is high in fiber content than the commonly consumed pulses and therefore can be considered in breeding for better nutritional qualities. Velvet beans has been shown to have anti Parkinson and neuroprotective effects, which may be related to its antioxidant activity. In addition anti-oxidant activities of m. pruriens has been also demonstrated in vitro by its ability to scaverage DPPH radical and reactive oxygen species.

CHAPTER ONE

1.0 INTRODUCTION

The genus Mucuna (velvet bean seed), belonging to the Fabaceae family,sub family Papilionaceae, includes approximately 150 species of annual and perennial legumes. Among the various under-utilized wild legumes, the velvetbeanMucuna pruriens (velvet bean seed) is widespread in tropical andsub-tropical regions of the world. It is considered aviable source of dietary proteins (Janardhanan, et. al.,2003; Pugalenthi, et. al., 2005) due to its high proteinconcentration (23–35%) in addition its digestibility,which is comparable to that of other pulses such assoybean, rice bean, and lima bean (Gurumoorthi, et. al.,2003). It is therefore regarded a good source of food.

The plant M.pruriens , widely known as “velvetbean,” is a vigorous annual climbing legume originallyfrom southern China and eastern India, where it wasat one time widely cultivated as a green vegetablecrop (Duke, 1981). It is one of the most popular greencrops currently known in the tropics; velvet beanshave great potential as both food and feed as suggestedby experiences worldwide. The velvet bean has beentraditionally used as a food source by certain ethnicgroups in a number of countries. It is cultivated in Asia,America, Africa, and the Pacific Islands, where its pods are used as a vegetable for human consumption, and its young leaves are used as animal fodder.

The plant has long, slender branches; alternate,lanceolate leaves; and white flowers with a bluishpurple,butterfly-shaped corolla. The

pods or legumesare hairy, thick, and leathery; averaging 4 inches long;are shaped like violin sound holes; and contain fourto six seeds. They are of a rich dark brown color, andthickly covered with stiff hairs. In India, the matureseeds of velvet bean.

CHAPTER TWO

2.0 LITERATURE REVIEW

Pu'galenthi, et al, (2005): Worked on velvet beans which were underutilized. They aim to study was to find out other utilization of velvet beans contain high level of carbohydrate protein, phenolics, tannins, L-Dopa protease inhibitors etc. They emphasized on underutilized legume for livestock and human being. The velvet bean are also used as food and feed worldwide. It has are many beneficial role in agriculture and many research has been conducted on its utilization due to some anti-nutrtional substances, utilization is limited.

Ganthi, et. zl; (2011): Studied on atmogapta is known as Mucuna pouriens, they worked on pharmaceutical studies of market seed sample of velvet beans. They compared commercially available seeds with wild, they analyzed seven species of mucuna pruriens, mucuna utilis coehinchonesis, mucuna atroporpurea, mucuna deeringiara canavalia, virosa & C-ensiformis. They found that mucuna deeringiana showed high content of L-Dopa, but cannavalia, virosa & C. ensifamis. Muccuna deeringana show low content of L-Dopa, virus a exhibited more CNS activity than other species finally they concluded that wild seeds have high content of L-Dopa % CAN more CNS activity than commercial available seeds.

Sundari, et. al. (2005): Studied on velvet beans for extraction and optimization, the objectives of this study was to employ mucuna pruriens for dyeing of leather (chrome tanned), response surface

methodology (RSM) was used for optimization. It is a statistical technique. The optimization was done by using Box-Behnken design, there were four independent variables such as temperature (30-70^0), time (30-90min feed to solvent, 1.3-1.7) and size of particle (0.25-0.75). It was found that 47% yield obtained under optimized condition. It was observed that dyed leather showed better color.

However Lawson et al; (2006) Claim that inter-cropping could help to supplies weed at sencondary growth afte ground cover has been fully established and refers to the use of velvet beans as a weed control strategy by forming a dense mat of vegetation to cut off sunlight to smother weeds. In this, Hammerton (2002) refers to the velvet beans as a legume species of choice. It seem to be very effective in suppressing and eliminating certain severe weeds, such as nut grass (cyperus rotondus). Bermuda grass (Cynodan dactylon) and cogan grass (imperatu cylindrical).

According to Hammerton (2002) the Ldopa substances allows impulses to be sent from one nerve cell to another in the brains, remarking that with Parkinson's disease insufficient dopamine is produced. The disease include symptoms such as trembling, stooped posture poor balance and slowness of the body movement.

Mortin-kilgour (2009) adds that the L-dopafrum velvet beans are safer and more effective for controlling Parkinson's disease a deficiency of the neurotransmitter dopamine mainly, because the array of other accompanying.

2.1 VELVET BEANS AS A TRADITIONAL MEDICINE

Velvet beans is a popular Indian medicinal plant,which has long been used in traditional AyurvedicIndian medicine, for diseases including parkinsonism(Sathiyanarayanan, et. al., 2007). This plant is widelyused in Ayurveda, which is an ancient traditionalmedical science that has been practiced in India sincetheVedic times (1500–1000 BC). Velvet beans isreported to contain L-dopa as one of its constituents(Chaudhri, 1996). The beans have also been employedas a powerful aphrodisiac in Ayurveda (Amin, 1996)and have been used to treat nervous disorders andarthritis (Jeyaweera, 1981). The bean, if applied as apaste on scorpion stings, is thought to absorb the poison(Jeyaweera, 1981).

The non-protein amino acid- derived L-dopa(3,4-dihydroxy phenylalanine) found in this underutilized legume seed resists attack from insects, and thus controls biological infestation during storage. Accordingto D'Mello (1995), all anti-nutritional compoundsconfer insect and disease resistance to plants. Further,L-dopa has been extracted from the seeds to providecommercial drugs for the treatment of Parkinson'sdisease. L-Dopa is a potent neurotransmitter precursorthat is believed, in part, to be responsible for the toxicityof theMucuna seeds (Lorenzetti et al., 1998). Antiepilepticandanti-neoplasticactivityofmethanolextractofM.pruriensshas been reported (Gupta et al., 1997).A methanol extract of MPseeds has demonstratedsignificant in vitro anti-oxidant activity, and there arealso indications that methanol extracts ofM. pruriensmay be a potential

source of natural anti-oxidants andanti-microbial agents (Rajeshwar et al., 2005).

All parts of velvet beans possess valuable medicinalproperties and it has been investigated in variouscontexts, including for its anti-diabetic, aphrodisiac,anti-neoplastic, anti-epileptic, and anti-microbialactivities (Sathiyanarayanan et al., 2007). Its antivenomactivities have been investigated by Guerrantiet al. (2002) and its anti-helminthic activity has beendemonstrated by Jalalpure (2007).M. pruriens has alsobeen shown to be neuroprotective (Misra and Wagner, 2007), and has demonstrated analgesic and anti-inflammatory activity (Hishika et al., 1981).

2.2 FUNCTIONAL COMPONENTS OF VELVET BEANS

In addition to the low levels of sulfur-containingphysiological and toxic factors may contribute to adecrease in their overall nutritional quality. Thesefactors include polyphenols, trypsin inhibitors, phytate,cyanogenic glycosides, oligosaccharides, saponins,lectins, and alkaloids. Polyphenols (or tannins) are ableto bind to proteins, thus lowering their digestibility.Phenolic compounds inhibit the activity of digestive aswell as hydrolytic enzymes such as amylase, trypsin,chymotrypsin, and lipase. Recently, phenolics have been suggested to exhibit health related functional propertiessuch as anti-carcinogenic, anti-viral, anti-microbial,anti-inflammatory, hypotensive, and anti-oxidantactivities.

Trypsin inhibitors belong to the group of proteinaseinhibitors that include polypeptides or proteins thatinhibit trypsin activity. Tannins exhibit weak interactionswith trypsin, and thus also inhibit trypsin activity.

Phytic acid [myoinositol-1,2,3,4,5,6-hexa(dihydrogenphosphate)] is a major component of all plant seeds, which can reduce the bioavailability of certainminerals such as zinc, calcium, magnesium, iron, andphosphorus, as well as trace minerals, via the formationof insoluble complexes at intestinal pH. Phytate-proteincomplexes may also result in the reduced solubility ofproteins, which can affect the functional properties ofproteins.

Cyanogenic glycosides are plant toxins that uponhydrolysis, liberate hydrogen cyanide. The toxic effectsof the free cyanide are well documented and affect awide spectrum of organisms since their mode of actionis inhibition of the cytochromes of the electron transportsystem (Laurena et al., 1994). Hydrogen cyanide(HCN) is known to cause both acute and chronictoxicity, but the HCN content of velvet seeds isfar below the lethal level. Janardhan et al. (2003) haveinvestigated the concentration of oligosaccharides invelvet seeds (m. Pruriens), and verbascose is reportedly theprincipal oligosaccharide therein (Siddhuraju et al.,2000). Fatty acid profiles reveal that lipids are a goodsource of the nutritionally essential linoleic and oleicacids. Linoleic acid is evidently the predominant fattyacid, followed by palmitic, oleic, and linolenic acids(Mohan and Janardhanan, 1995; Siddhuraju et al.,1996). The nutritional value of linoleic acid is due to

itsmetabolism at tissue levels that produce the hormonelikeprostaglandins.Theactivityoftheseprostaglandinsincludes lowering of blood pressure and constrictionof smooth muscle.Phytohemagglutinins (lectins) aresubstances possessing the ability to agglutinate human erythrocytes.

The major phenolic constituent of velvet beans was found to be L-dopa (5%), along withminor amounts of methylated and non-methylatedtetrahydroisoquinolines (0.25%) (Sidhuraju et al., 2001; Misra and Wagner, 2004). However, in additionto L-dopa, 5-indole compounds, two of which wereidentified as tryptamine and 5-hydroxytryptamine, werealso reported in velvet seed extracts (Tripathi andUpdhyay, 2001). Mucunine, mucunadine, prurienine,and prurieninine are four alkaloids that have beenisolated from such extracts (Mehta and Majumdar, 1994).

2.3 PHARMACOLOGICAL EFFECTS OF VELVET BEANS EXTRACTS

All parts of the Mucuna plant possess medicinalproperties (Sathiyanarayanan and Arulmozhi, 2007). In vitro and in vivo studies on M. pruriens extractshave revealed the presence of substances that exhibit awide variety of pharmacological effects, including anti-diabetic, anti-inflammatory, neuroprotective and antioxidant properties, probably due to the presence of L-dopa, a precursor of the neurotransmitter dopamine (Misra and Wagner, 2007). It is known that the main phenolic compound ofMucuna seeds is L-dopa(approximately 5%) (Vadivel and Pugalenthi, 2008).Nowadays,Mucuna is widely

studied because L-dopa isa substance used as a first-line treatment for Parkinson'sdisease. Some studies indicate that L-dopa derived from velvet seed (M. pruriens) has many advantages over synthetic L-dopawhen administered to Parkinson's patients, as syntheticL-dopa can have several side effects when used formany years.

In small amounts (approximately 0.25%) L-dopacorresponds t o met hylated and non-methylatedtetrahydroisoquinoline (Siddhuraju and Becker, 2001;Misra and Wagner, 2004). These substances are present in theMucuna roots, stems, leaves, and seeds. Othersubstances are present in different parts of the plant,among which areN,N-dimethyl tryptamine and someindole compounds (Tripathi and Updhyay, 2001).Alcoholic extracts of the seeds were shown to havepotential anti-oxidant activity in invivo models of lipidperoxidation induced by stress (Tripathi and Updhyay,2001). On the other hand, Spencer et al. (1996) havereported that the pro-oxidant and anti-oxidant actionsof L-dopa and its metabolites promote oxidative DNAdamage and could also be harmful to tissues damagedby neurodegenerative diseases, namely parkinsonism.Moreover, a study usingin vitro models revealed thatL-dopa significantly increases the levels of oxidizedglutathione in rat brain striatal synaptosomes (Spinaet al. , 1988). The observed depletion of reducedglutathione (GSH) could be due to the generation ofreactive semiquinones from L-dopa (Spencer et al,1995).

Table 1. Pharmacological activity of Mucuna pruriens and its compounds

Pharmacological activities	Plant component	Extract	Material/ compound	References
anti-venom	plant seeds	water	proteins (gpMuc)	Guerranti, 2002; Guerranti, 2004; Guerranti, 2008
anti-microbial	plant leaves	methanol	tannins, alkaloids, L-dopa	Sofowora, 1982; Mandal, 2005; Ogundare and Olorunfemi, 2007
neuroprotective	plant seeds, whole plant	ethanol/water (1:1)	L-dopa, amino acids, alkaloids	Kulhalli, 1999; Misra and Wagner, 2007; Misra, 2007
		n-propanol	isoquinolines, alkaloids	
anti-diabetic	plant seeds	ethanol/water (1:1)	cyclitols, oligosaccharides	Horbovitz, 1998; Larner, 1998; Ortmeyer, 1995

1 N-terminal amino acid sequence of protein spots of gpmuc fraction

Spot No.	aa											
	1	2	3	4	5	6	7	8	9	10	11	12
A												
gP1	K	D	D	K	E	P	V	K	D	T	D	G
gP2	K	D	D	K	E	P	V	K	D	T	D	G
gP4	K	D	D	K	E	P	V	K	D	T	D	G
B												
gP3	K	D	D	K	E	P	V	R	D	T	K	K
C												
gP5	K	N	D	G	E	L	V	R	D	T	Y	G
gP7	K	N	D	G	E	L	V	K	D	T	Y	G
gP6	K	N	D	G	E	L	V	K	D	T		

2.4 PROTECTIVE EFFECT OF VELVET SEEDS (MUCUNA PRURIAS) AGAINST SNAKE VENOM POISONING

Velvet beans seed is one of the plants that have beenshown to be active against snake venom and, indeed,its seeds are used in traditional medicine to prevent thetoxic effects of snake bites, which are mainly triggeredby potent toxins such as neurotoxins, cardiotoxins,cytotoxins, phospholipase A_2 (PLA_2), and proteases(Guerranti et al., 2002). In Plateau State, Nigeria, theseed is prescribed as a prophylactic oral anti-snakebiteremedy by traditional practitioners, and it is claimedthat when the seeds are swallowed intact, the individualsnake bite (Guerranti et al., 2001). The mechanismsof the protective effects exerted by Velvet beans seedaqueous extract (MPE), were investigated in detail, ina study involving

the effects of Echis carinatus venom(EV) (Guerranti et al., 2002). In vivo experimentson mice showed that protection against the poison isevident at 24 hours (short-term), and 1 month (longterm) after injection of MPE (Guerranti et al., 2008).MPE protects mice against the toxic effects of EV viaan immune mechanism (Guerranti et al., 2002). MPEcontains an immunogenic component, a multiformglycoprotein, which stimulates the production ofantibodies that cross-react with (bind to) certain venomproteins (Guerranti et al., 2004). This glycoprotein,called gpMuc (see Table 1), is composed of sevendifferent isoforms with molecular weights between 20.3and 28.7 kDa, and pI between 4.8 and 6.5 (Di Patrizi et al., 2006).

It is likely that one or more gpMuc isoform is analogous in primary structure to venom PLA. Thepresence of at least one shared epitope has beendemonstrated with regard to MP seeds and snake venom.These cross-reactivity data explain the mechanism ofthe long-term protection conferred by MP, and confirmthat certain plant species contain PLA_2-like proteins,which are beneficial for plant growth, and are involvedin important processes (Lee et al., 2005). In addition, (velvet beans seeds) contain protein and non protein componentsthat are able to directly inhibit the activity of proteasesand PLA_2 (phospholipase A_2), and are responsible for short-term protection.In fact, MPE contains protease inhibitors that are activeagainst snake venom, in particular a gpMuc isoformsequence also found in a “Kunitz type” trypsin inhibitorcontained in soy. Two-dimensional gel electrophoresishas been used to separate the seven gpMuc isoforms, inorder to perform N-terminal analysis of each individualisoform. The sequences obtained are shown in Figure 1.According to their sequences, we can group the isoformsat positions 1, 2, and 4 on the gel, which are identical in12/12 aa. The isoform at position 3 is identical to thoseaforementioned, with regard to

the first 10 aa, and thoseat positions 5, 6, and 7 differ from those at positions 1,2and 4 by just 3 aa (Guerranti et al., 2002; Scirè et al.,2011; Hope-Onyekwere et al., 2012). On the other hand,the direct inhibitory action of MPE is probably causedby L-dopa, the main bioactive component, which acts insynergy with other compounds.

2.5 NEUROPROTECTIVE EFFECT OF VELVET BEANS SEED

In India, the seeds of M. pruriens have traditionally been used as a nervine tonic, and as an aphrodisiac for male virility. The pods are anthelmintic, and the seeds are anti-inflammatory. Powdered seeds possess antiparkinsonism properties, possibly due to the presence of L-dopa (a precursor of neurotransmitter dopamine). It is well known that dopamine is a neurotransmitter. The dopamine content in brain tissue is reduced when the conversion of tyrosine to L-dopa is blocked. L-Dopa, the precursor of dopamine, can cross the blood-brain barrier and undergo conversion to dopamine, restoring neurotransmission (Kulhalli, 1999). Good yields of L-dopa can be extracted from M. pruriens seeds (Table1) with EtOH-H O (1:1), using ascorbic acid as a protector (Misra and Wagner, 2007). An n-propanol extract of M. pruriens seeds yields the highest response in neuroprotective testing involving the growth and survival of DA neurons in culture. Interestingly, n-propanol extracts, which contain a negligible amount of L-dopa, have shown significant neuroprotective activity, suggesting that a whole extract of M. pruriens seeds could be superior to pure L-dopa with regard to the treatment of parkinsonism.

2.6 ANTI-MICROBIAL PROPERTIES OF VELVET BEANS SEED

Various parts of certain plants are known to containsubstances that can be used for therapeutic purposesor as precursors for the production of useful drugs(Sofowora, 1982). Plant-based anti-microbials representa vast

untapped source of medicines and furtherinvestigation of plant anti-microbials is needed. Antimicrobialsof plant origin have enormous therapeuticpotential. Phytochemical compounds are reportedlyresponsible for the anti-microbial properties of certainplants (Mandalet al ., 2005). While bioactive compoundsare often extracted from whole plants, the concentrationof such compounds within the different parts of the plantvaries. Parts known to contain the highest concentrationof the compounds are preferred for therapeutic purposes.Some of these active components operate individually, others in combination, to inhibit the life processes ofmicrobes, particularly pathogens. Crude methanolicextracts ofM. pruriens leaves have been shown to havemild activity against some bacteria in experimentalsettings (Table 1), probably due to the presence ofphenols and tannins (Ogundare and Olorunfemi, 2007).Further studies are required in order to isolate thebioactive components responsible for the observed antimicrobialactivity.

2.7 ANTI-DIABETIC EFFECT OF VELVET BEANS SEED

Using a combination of chromatographic and NMR techniques, the presence of d-chiro-inositol and itstwo galacto-derivatives, O-a-d-galactopyranosil-(1→2)-dchiro-inositol(FP1) and O-a-d-galactopyranosil-(1→6)-O-a-d-galactopyranosil-(1→2)-Dchiro-inositol(FP2),was demonstrated inM. pruriens seeds (Donati et al ., 2005). Galactopyranosyl d-chiro-inositols arerelatively rare and have been isolated recently fromthe seeds of certain plants; they constitute a minorcomponent of the sucrose fraction ofGlycine max(Fabaceae) and lupins, and a major component of Fagopyrum esculentum (Polygonaceae) (Horbovitz etal., 1998). Although usually ignored in phytochemicalanalyses conducted for dietary purposes, the presence ofthese cyclitols is of interest due to the insulin-mimeticeffect

of d-chiro-inositol, which constitutes a novelsignaling system for the control of glucose metabolism(Larneret al ., 1998; Ortmeyer et al., 1995). Accordingto Anktar et al., (1990),M. pruriens seeds used at adose of 500 mg/kg reduced plasma glucose levels.These and other data demonstrated that the amount ofseeds necessary to obtain a significant anti-diabeticeffect contain a total of approximately 7mg of dchiroinositol(including both free, and that derived from thehydrolysis of FP1 and FP2). The anti-diabetic propertiesof M. pruriens seed EtOH/HO 1:1 extract are mostlikely due to dchiro-inositol anditsgalacto-derivatives(Table 1).

2.8 ANTI-OXIDANT ACTIVITY OF VELVET

Free radicals that have one or more unpairedelectrons are produced during normal and pathologicalcell metabolism. Reactive oxygen species (ROS) reactreadily with free radicals to become radicals themselves.Anti-oxidants provide protection to living organismsfrom damage caused by uncontrolled production ofROS and concomitant lipid peroxidation, proteindamage and DNA strand breakage. Several substancesfrom natural sources have been shown to contain anti-oxidants and are under study. Anti-oxidant compoundssuch as phenolic acids, polyphenols, and flavonoids,scavenge free radicals such as peroxide, hydroperoxideor lipid peroxyl, and thus inhibit oxidative mechanisms.Polyphenols are important phytochemicals due to theirfree radical scavenging andin vivo biological activities(Bravo, 1998); the total polyphenolic content has beentested using Folin-Ciocalteau reagent. Flavonoids aresimple phenolic compounds that have been reportedto possess a wide spectrum of biochemical properties, including anti-oxidant, anti-mutagenic and anticarcinogenicactivity (Beta et al., 2005). The hydrogendonating ability of the methanol extract ofM. prurienswas measured in the presence of 1,1-diphenyl-2picryl-

hydrazyl(DPPH) radical. In a recent study,Kottai Muthu et al. (2010) found that ethylacetate andmethanolic extract of wholeM. pruriens plant (MEMP),which contains large amounts of phenolic compounds,exhibits high anti-oxidant and free radical scavengingactivities. These in vitro assays indicate that this plantextract is a significant source of natural anti-oxidant,which may be useful in preventing various oxidativestresses. It has been reported (Ujowunduet al ., 2010)that methanolic extracts of M. pruriens leaves have numerous biochemical and physiological activities, andcontain pharmaceutically valuable compounds (Table 1).

2.9 POSSIBLE USAGE OF VELVET BEANS FOR SKIN TREATMENTS

The skin is one of the main targets of severalexogenous insults such as UV radiation, O_3, andcigarette smoke, and all of these exert toxicity via theinduction of oxidative stress (Valacchi et al., 2000).Several skin pathologies, such as psoriasis, dermatitis,and eczema, are related to increased oxidative stressand ROS production (Briganti and Picardo, 2003),and research investigating novel natural compoundswith anti-oxidant proprieties is an expanding field. Asmentioned above, certain plant-derived compoundshave been an important source of traditional treatmentsfor various diseases, and have received considerableattention in more recent years due to their numerouspharmacological proprieties.

Recent preliminary studies from our group have shown that human keratinocytes treatedwith a methanolic extract from MP leaves exhibitdownregulation of total protein expression. In addition,treatment

with MP significantly decreased the baseline levels of 4HNE present in human keratinocytes(Lampariello et al., 2011). This preliminary studysuggests that evaluating the effect that topical MPmethanolic extract treatment may have on skin diseases, the mechanisms involved in such effects.

2.10 AIM AND OBJECTIVE

The aim of the study is tocompare the proximate and mineral composition of Indian legume seed (white and black seeds of velvet beans).

The specific objective of the study were:

1. To determine the proximate composition of black and white velvet beans
2. To determine the mineral composition of black and white velvet beans

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1.1 COLLECTION AND PREPARATION OF SAMPLES

White and black mature seeds of velvet beans (mucunal pruriens) were collected from Ara village, Iloin, Kwara State, Nigeria. After thoroughly drying in the sun, the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds and foreign material were stored in air tight plastic jars at room temperature (25^0C).

50g of white and black velvet beans is then weigh with weighing balance. The seeds is then grind with grinder. The powder form of white and black velvet beans is then taken to the laboratory.

3.1.2 APPARATUS

- Aluminum dishes
- Oven
- Desiccators
- Weighing balance
- Platinum crucible
- Furnace
- Cotton wool
- Soxhlet flask and flask
- Extractor
- Heating mantle
- Rotary vacuum evaporator

- Filter paper
- Fume cupboard
- Conical flask
- Flutter funnel
- Test tube
- Corvette
- Incubator
- Visible/ultra violet spectrophotometer
- X-ray fluorescence
- Micro pipette

3.1.3 REAGENT

- Water (H_2O)
- Sulphuric acid (H_2SO_4)
- Hydrochloric acid (HCl)
- Cupper tetra oxosulphate ($CuSO_4$)
- Sodium hydroxide (NaOH)
- Boric acid
- Methyl red indicator
- Ammonia (NH_3)
- Calcium reagent
- Potassium reagent
- Acid reagent
- Sodium color reagent
- Phosphorus reagent
- Sodium tetro oxosulphate (Na_2SO_4)

3.2.1 PROXIMATE ANALYSIS

3.2.1.1 DETERMINATION OF MOISTURE CONTENT

This method is based on moisture evaporation. Here the aluminum dishes were washed dried in oven and in desiccators for cooling. The weight of each dish was taken. 5.0g of ground samples of were weighted into a sterile aluminum dish, weight of dish and weight of un-dried sample (in duplicate) were taken. This was transferred into an oven set at 80^0c for 2h and at 100^0 for 3 respectively. This was removed and cooled in desiccators. Then the weigh it was measured using a measuring scale balance. It was transferees back into the oven for another one or and then reweighted. The process continues until a constant weight was obtained. The difference in weight between the initial weight and the constant weight gained represents the moisture content.

Calculation: the loss in weight multiplied by 100 over the original weight is percentages moisture content.

Moisture content (g/ 100g) = loss in weigh (W2-W3)/ (W2-W1)x 100

Where WI = initial weight of empty crucible W2= weight of crucible + food before drying W3 = final weight of crucible + food after drying.

% Total solid (Dry matter (%)= 100-moisure (%)

(AOAC 2005).

3.2.1.2 ASH CONTENT

The ash presents the inorganic component (Minerals) of the sample after all moisture has been removed as well as the organic material. The method is destructive approach based on the

decomposition of all organic matter such that the mineral elements may be lost in the process. Twenty grams (20g) of each of the samples were weighted into a clean dried and cooled platinum crucible. It was put into a furnace set at 550 DC and allowed to blast for 3h. it was then brought out and allowed to cool inn desiccators and weighed again.

Calculation: Percentage weight is calculated as weight of as multiplied by 100 over original weight of the samples used.

Ash content= (weight of ash/weight of original sample used) x 100.

Where WI - weight of empty crucible, W2 = weight of crucible food before drying and or ashing W3= weight of crucible + ash. (AOAC 2005).

3.2.1.3 DETERMINATION OF LIPID CONTENT

The method employed was the soxlet extraction technique described by Shir law, (1967). 15 g of the sample were weighted and carefully placed inside a fat free thimble. This was covered with cotton wool to avoid the loss of sample. Loaded thimble was put in the soxhlet extractor, about 200 ml of petroleum ether were poured into a weighted far free soxhlet flask and the flask was attached to the extractor. The flask was placed on a heating mantel so the petroleum ether in the flask refluxed. Cooling was archived by a running tap connected to the extractor for at least 6hrs after which the solvent was completely siphoned into the flask. Rotary vacuum evaportator was used to evaporate the solvent leaving behind the extracted lipids in the soxhlet. The flask was removed from the evaporator and dried to a constant weight in the oven at 60^{o}C. the flask was then cooled In a desiccators

and weighed. Each determination was done in triplicate. The amount of fat extracted was calculated by difference.

Each extracts (100g) dry matter= (weight of extracted lipids/weight of dry dample) x 100 (AOAC2005)

3.2.1.4 PROTEIN DETERMINATION

Total protein was determined by the kjeldahl method as modified by Williams (1964). The analysis of a compound of its protein content by kjeldahl method is based upon the determination of the amount of reduced nitrogen present. About 20g of the samples were weighed into a filter paper and put into a kjedahl flask, 10 tablets of Na_2SO_4 were added with 1g of $CuSO_4$ respectively. Twenty milliters (20ml) of conc H_2SO_4 were added and then digested in a fume cupboard until the solution becomes colorless. It was cooled overnight and transferred into a 500ml flat bottom flask with 200ml of water. This was then cooled with the aid of packs of ice block. About 60 to 70ml of 40% of NaOH were poured into the conical flask which was used as the receiver with 50ml of 4% boric acid using 3days of screend methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporates. Titration was done in the receiver with 0.01M HCl until the solution become colorless.

Calculation: The percentage protein is calculated as follows.

Where Vs –vb x0.014cl x N acid (6.25 x 100 original w.t of sample used

Where Vs = volume (ml) of acid required to titrate sample

Vb = volume of acid required to titrate blank Nacid = normality of acid (AOAC 2019).

3.2.15 DETERMINATION OF CRUDE FIBER

The bulk of roughages in food is referred to as fiber and is estimated as crude fiber. Twenty grams (20g) of the different sample were defatted with dietyl ether for 8h and boiled under reflux for exactly 30 min with 200 ml of 1.25% H_2S0_4. It was then filtered through cheese cloth on a fluther funnel. This was washed with boiling water of completely become the acid. The residue was then boiled in a round bottomed flask with 200 ml of 1.35 sodium hydroxide (NaOH) for another 30min and filtered through previously weighted couch crucible. The crucible was then dried with samples in an oven at 100^{o}C, left to cool in a desiccators and later weighted. This was later incinerated in a muffle furnace at 600^{0}C for 2 to 3 in and later allowed to cool in a desecrator and weighed, (AOAC 2019).

Calculation = Weight of fiber = (C2-C3)y

% fiber = C2-C3 x 100/Wt. of original sample

3.2.1.6 CARBOHYDRATE DETERMINATION

Available carbohydrate (%) Protein (%) + Moisture (%) + Ash (%) + fibre (%) + Fat (%).

Energy caloric Value (KJ /100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7).

3.2.2 DETERMINATION OF MINERAL ANALYSIS

2.2g of ash sample was put in a test tube and was digested with a mixture of 5ml of 10% HCl. The sample was boil at 90^{0}C, after boiling 5ml of H_2O was added. The sample is called supernatant 12.5ml of supernatant was added with 500nl of reagent and mix. It is then

incubate for 5minutes. Each mineral element were analysed with each reagent.

All mineral element (calcium, phosphorus, potassium except sodium were analyzed from a acid-digested sample by a ultra violent/visible spectrophotometer according to their wave length.

Sodium was analysed by putting 50ml of supernatant in a test tube 1000ml of acid reagent was added to it. Sodium color reagent was also added and incubate for 5minutes. The acid-digested sample was read in a ultra violent/visible spectrometer.

Wave length of calcium is 578nm

Wave length of phosphorus is 340nm

Wave length of potassium is 500nm

Wave length of sodium is 500nm

CHAPTER FOUR

4.1 RESULT

TABLE 4.1 RESULT OF PROXIMATE ANALYSIS OF BLACK VELVET BEANS

Component	Black seed
Moisture %	9.64± 0.05
Ash %	4.97±0.09
Carbohydrate %	53.28±0.78
Crude lipid	4.79±0.51
Crude fiber	1.24±0.04
Crude protein	26.08±0.09
Calorific value CKj/100g	1505.79±7.64

TABLE 4.2 RESULT OF PROXIMATE ANALYSIS OF WHITE VELVET BEANS

Component	White seed
Moisture %	10.20±0.0024
Ash %	4.86±0.010
Carbohydrate %	53.63±0.10
Crude lipid	6.60±0.05
Crude fiber	1.28±0.07
Crude protein	23.43±0.09
Calorific value CKj/100g	1535.85±2.16

TABLE 4.3 RESULT OF MINERAL ANALYSIS OF BLACK VELVET BEANS

Component	Black seed
Sodium (ppm)	12.68±1.45
Calcium	1.31±0.01
Potassium	0.37±0.001
Phosphorus	0.11±0.005
Na/K	0.05
Ca/P	2.28

TABLE 4.4 RESULT OF MINERAL ANALYSIS OF WHITE VELVET BEANS

Component	Black seed
Sodium (ppm)	32.48±0.15
Calcium	1.54±0.012
Potassium	0.033±0.005
Phosphorus	0.034±0.005
Na/K	0.08
Ca/P	1.51

4.2 DISCUSSION

The proximate composition of the white and black seeds of velvet beans are shown in table 4.1 proximate analysis result showed that black seed has higher crude protein content (26.08%) whereas the protein content of white seed was (23.43%). These selected samples seeds make the legumes a good source of nutrition. High fiber content was observed in white weeds. It is observed that white black seed of selected samples have moderate range of carbohydrate. The white seeds recorded high energy (1.535.85kj/1000g).

The proximate composition of velvet bean seeds are shown in table 1, moisture content is 10.20g/100g and 9.64/100mg of white seeds accession and black seed respectively. Crude proteins and carbohydrates are the major chemical constituents of the legume sample. The crude protein content was significant at 23.43g/100g, and higher than commonly consumed legumes in India such as vigna ungiculata (Ayssiwede et al; 2011) wild legumes as Rhychosiacana, R. Fillips, R. rufescens and R. suaveolens and species of vigna (Kalidass and Mohan, 2012a, b). The total dietary fiber content is found to be higher in the seeds of both the accessions of mucuna pruriens.

The ash content of investigated mucuna beans (above 490) would be important to the extend that it contains the nutritionally important mineral element similar values were reported in cassia obtusifolia (vigjaya kumara et al; 1993). The food energy value was calculate to be (1535.85kj/100g) base on the crude protein, crude lipid &NEE. The high caloric values were due to its high fat content. This is comparable

to previous studies, in the seeds of canavalia gladiate (vadivel and Janardhana, 2004). Both seed of velvet beans investigated have higher energy than the reported value for pulses Rhychosia cana, R. Fillips and R. DUarelens (Kalidass and Mohan, 2012a) and Canavalia ensiformis (Doss al; 2011).

Analysis showed that both had significantly different ($P<0.05$) levels of minerals content, (table 4.3 and 4.4). higher concentration of sodium (32.48ppm), calcium (4.54ppm), phosphorus (0.033pm) are in white seed while as black seed possessed higher quantum of potassium. The minerals composition of both seeds, expressed in ppm dry matter (DM) showed that is rich in sodium, calcium and fairly rich in potassium and phosphorus. The ratio of sodium to potassium (Na/K) and calcium to phosphorus (Ca/P) are show in (table 4.3&4.4). The Na/K ratio in the beans can be useful for prevention of high blood pressure. Food is considered “good” in the present study, the Ca/p ratio is 1:51 for white sees and 2.28 for black seeds.

CHAPTER FIVE

5.1 CONCLUSION

Velvet beans (mucuna pruriens) is an exceptional plant from the present study it can be conclude that it is a good source of food as it is rich in crude, protein and starch content on the other hand, it also contain various anti nutritional factors such as protease inhibitor, total phenolic, oligosaccharide and some cyclitols with anti-diabetic effect in facts all pats of Mucuna plant possess medicinal properties. The main phenolic compound is L-dopa (15%) and M. pririas seed contains some conponents that are able to inhibit snake venoms, in addition, methanolic extract of m. pruriens leave has demonstrated antimicrobial and antioxidants activities in the presence of bio-active compound such as phenols, polyphenols and tannis and preliminary studies on keratinocytes support its possible topical usage to treat redox driven skin disease.

From the study it is evident that velvet beans is a potential source of protein supplement for livestock as well as in human food and also it serve as good source of minerals for bone formation.

5.2 RECOMMENDATION

- From the findings obtain from this work the following recommendation can be made. All the part of mucuna plant process medicinal properties (anti-venom, anti-microbia, neuroprotective, anti-diabetic) pharmaceutical industries should be encourage to use velvet beans seed to produce various drugs and food supplement.

- Fermented mucuna leaf meals at 25% replacement level of soya bean meal for better performance is suggested.
- The return on investment is higher when mucuna leaf meal is sued to replace soya bean meal or 25% inclusion level in claries gariepinus fingerlings.

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