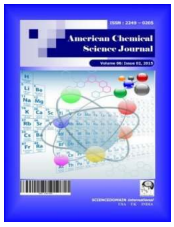

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Antioxidant, Anti-inflammatory and Antibacterial Activities of the Seeds of *Mucuna pruriens (UTILIS)*

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Authors' contributions

This work was carried out in collaboration between all authors. Author RIU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CNM, KOA, CEI and ICI managed the analyses of the study. Authors AAA and PCIN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To ascertain the antioxidant, anti-inflammatory and antibacterial activities of the extracts of seeds of *Mucuna pruriens (utilis).*
Study Design: The study was designed to test the inhibitory ability of the plant extract on edema and human pathogens and to test its antioxidant activity.
Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri. Imo state Nigeria, between March 2013 to September 2014.
Methodology: The seeds of *M. pruriens* were cracked, the testa were removed and the seeds were milled into fine powder with Thomas Willey milling machine and dissolved in ethanol for 48 hrs, this was filtered. The filtrate was concentrated with Rota evaporator at 40℃ and then used for analysis. The effects of *Mucuna pruriens* (*utilis*) on the acute and chronic phases of inflammation were studied in carrageenan and formalin induced paw edema respectively. The anti-edema effect

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of these seeds was compared with diclofenac potassium 10 mg/kg orally. The antibacterial activity was performed by filter paper disc diffusion technique. The antioxidant activity of the seeds of *M. pruriens* was evaluated by reducing power test.

Results: Administration of *M. pruriens* (10 mg/kg) and diclofenac potassium (10 mg/kg) decreased the inflammation at the rate of 9.8% and 62.0% respectively and increase in dosage of *M. pruriens* (50 mg/kg) showed a decrease at the rate of 47.80% for acute inflammation and 38.80% for chronic inflammation. Thus the crude extracts exhibited significant activity in rats. The seeds were found to have high antioxidant activities. Extracts of the seeds of *Mucuna pruriens* exhibited strong antibacterial activities on all the tested organisms, *Staphylococus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli*. The lowest concentration of the seed extract that inhibited the visible growth of an organism after overnight incubation was 12.5 mg/ml for all tested organisms except for *Klebsiella pneumonia which was* 25.00 mg/ml. This indicates that *M. pruriens* seeds may be used against bacterial infections, inflammation and as antioxidant in herbal medical practice.

Keywords: Antioxidant; anti- inflammatory; antibacterial; therapeutic agents.

1. INTRODUCTION

Medicinal plants contain numerous biologically active compounds which have shown considerable pharmacological activities such as antioxidant, antimicrobial, anti inflammatory, anticancer, antiviral, anti-allergic and vasodilatory properties [1,2]. Such medicinal plants include *Mucuna pruriens*. *Mucuna pruriens* (utilis) also known as velvet bean is a vigorous annual climbing legume which originally came from southern China and eastern India. It grows up to 30 ft. 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 [3]. *Mucuna pruriens* is called 'Agbara' in Igbo land (Eastern Nigeria). Two species of *Mucuna pruriens* are recognized locally, the non edible one with long stinging hairs on the pod which causes an intensely itchy dermatitis on human contact and the edible one with non stinging silky hairs known by the common English name "velvetbean,". *Mucuna pruriens* (*utilis*) is extensively used in herbal medicine and as food. Nutritional importance of *Mucuna pruriens* seeds as a rich source of protein supplement and carbohydrate has been well documented [4] who studied the proximate composition of *Mucuna pruriens* (utilis) found high protein (26.40%), [5] also reported high protein (25.3%), minerals and vitamins in the seeds of *M. pruriens.* [6] studied the quantitative composition of amino acids in *M. pruriens* and discovered that the quantity of amino acids in the seeds of *M. pruriens* is comparable to that of soyabean, thus the flour has been recommended to be incorporated into cereals and root/tuber food products to improve their nutritional quality [7,5]. 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 [8,9].

Traditionally, *M. pruriens* plant is used in the treatment of various diseases such as parkinson disease. The roots are used to expel several species of worms except tape worm and to treat abdominal discomfort, cholera, snake bite, ulcer, toothache and as a laxative [10]. The seeds of *M. pruriens* are used in traditional medicine to treat diabetes, enhance sexual stamina, sensation and libido in aging individuals, and also in treatment of disorders of male and female reproductive systems [11]. *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 [12].

Some compounds have been isolated from the seeds of *Mucuna pruriens* (Utilis) which include a steroid, Estra – $2^{//}$ – 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 to increase libido in both men and women and as anti- depressant [13]. Also another compound 5,7 – dimethoxy, $4^{/}$– propoxyflavone was isolated, this compound suggested the reason why *M. pruriens* seeds is used in traditional medicine to treat elephantiasis, bacterial infections and to heal wounds [14]. [15] carried out GC-MS analysis on the seeds of *M. pruriens* and reported that the seeds contain phytochemicals which include alpha-amyrin,

beta-amyrin, 4, 4-dimethylandrostan-3-yl acetate e.t.c. Alpha-amyrin and beta-amyrin are pentacyclic triterpenoids which are known to have anti hyperglycemic and hypolipidemic activities. [16] reported that both alpha-amyrin and beta-amyrin have anti – inflammatory activities. They were reported to retard acute inflammation in rats. 4, 4-dimethylandrostan-3-yl acetate is a natural hormone that functions predominantly as a metabolic intermediate in the biosynthesis of the androgen and estrogen sex hormone, they play major sex – related roles [17,18].

In spite of the various uses of this plant as food, feed and medicine, pharmacological activity of *M. pruriens* plants are yet to be fully documented thus this study was focused on anti-inflammatory and antioxidant and antibacterial activities of extract of seeds of *M. pruriens* in order to ascertain its usefulness as anti- inflammatory, antioxidant and antibacterial agents.

2. MATERIALS AND METHODS

2.1 Plant Materials

The seeds of *M. pruriens* were harvested from the field of National Root Crops Research Institute Umudike. Authentication of plant materials was done by Dr A. Nmeregini of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.

2.1.1 Sample preparation

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.

2.2 Antioxidant Activities of the Seeds of *M. pruriens*

The antioxidant activity of the seeds of *M. pruriens* was evaluated by reducing power test. The reducing property was determined by assessing the ability of the sample extracts to reduce $FeCl_3$ solution as described by [19].

200 mg of the powdered seeds of *M. pruriens* was weighed and macerated in 50 ml of methanol for 24 hours. This was filtered. Then 1.0, 2.0, 3.0, and 4.0 ml of each methanol extract were put in a separate test tubes. 0.5 ml of phosphate buffer (0.2 M, pH 6.6), and 2.5 ml of potassium hexacyanoferrate solution (1%) were added to each test tube and reacted for 20 mins at 50℃. The tubes were cooled immediately by using crushed ice and an aliquot of 0.5 ml of Trichloroacetic acid (10%) was added in all the tubes. These were centrifuged at 3000 rpm for 10 min. The absorbance of the solutions were read at 700 nm with spectrometer. Increased absorbance of the reaction mixture indicated an increased reducing power.

2.3 Anti-inflammatory Activities of *M. pruriens*

The method described by [20] was used in this determination.

Anti-inflammatory activity of *M. pruriens* seeds was determined by carrageenan-induced acute and formalin-induced chronic inflammatory models in albino rats. The samples were administered orally.

2.3.1 Carrageenan-induced paw edema

18 male albino rats were divided into three groups. Six animals in each group, the inflammation was induced by single sub-plantar injection of 20 µl of freshly prepared 1% carrageenan suspension in normal saline. Group one was treated with carrageenan alone and served as control. Groups two and three received *M. pruriens* all at concentration of 10 and 50 mg/kg body weight orally/hour before the carrageenan injection. The samples were presolubilized in 0.2% dimethyl sulfoxide and a fine suspension was prepared in phosphate buffered saline. Group four was administered with reference drug diclofenac potassium also orally 1 hour before carrageenan injection. The paw thickness of animals in all groups was measured using vernier calliphers before and 3 hours after carrageenan injection.

2.3.2 Formalin-induced paw edema

The same procedure was adopted as described above except that single dose of 0.02 µl of formalin (2%) was used to induce inflammation. The samples were administered once daily for six consecutive days. In the above two models, the degree of edema formation was determined as increase in paw thickness. In the case of acute anti-inflammatory activity, paw thickness was measured once daily for six days. The increase in paw thickness and percent inhibition were calculated as follows:

$$\% \text{ Inhibition} = \frac{Pc - Pt}{Po} \times \frac{100}{1}$$

Where : Pt = Paw thickness at time, t; Po = Initial paw thickness; Pc = Increase in paw thickness of the control group; Pt = Increase in paw thickness of the treatment groups.

2.4 Anti–bacterial Evaluation of the Seeds *M. pruriens*

2.4.1 Preparation of extracts

The test solution of each extract was prepared by dissolving 100 mg of the plant extracts separately in 1.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml.

2.4.2 Micro-organisms

The bacteria organisms used were *Staphylococus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli*. All the organisms were obtained from the stock culture of the Federal Medical Center, Umuahia. Cultures were brought to laboratory conditions by resuscitating the organisms in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37℃ for 24 hours [21].

2.4.3 Antibacterial assay

The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (whatman No1, 6 mm diameter) were placed in glass petri dishes and sterilized in hot air oven. The media (10 g nutrient agar in 200 ml distilled water, auto-claved at 115℃ for 30 minutes) was cooled to 50℃. The sterile nutrient agar media were poured into the sterile petri dishes and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 ml of plant extracts and standard- Ciprofloxacin. Discs with DMSO (100 mg/ml) served as a control [21].

The discs were used after drying them in an incubator at 40℃ to remove any trace of solvent. Discs were introduced onto the surface of the medium. The plates were incubated at 37℃ for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotic to minimize error and the average of these values were tabulated in Table 2.

2.5 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extracts was determined by incorporating constant volumes (0.2 ml) of each dilution of the extract into the perforated discs on a seeded nutrient agar plate as described in the antimicrobial susceptibility testing section. 100 mg of each extract was dissolved in 1 ml of DMSO to obtain 100 mg/ml. This 100 mg/ml concentration was then doubly diluted in DMSO to obtain concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml.

2.6 Statistical Analysis

All values are expressed as mean ± S.D. Statistical analysis were performed by Student's *t*-test. The values of p lower than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Antioxidant activity of the methanolic extracts of seeds of *M. pruriens* is as shown in Fig. 1. The extracts showed a good antioxidant activity with a dose dependent response. There was a corresponding increase in the reducing ability with increase in concentration of the extracts. Antioxidants are substances that prevent damage to cells caused by free radicals.

Table 1. Effect of methanol extract of *M. pruriens* seeds on carrageenan and formalin induced paw oedema in rats

Treatment	Dose(mg/kg)	% Inhibition
Carrageenaan (control)	0.1 ml	-
Diclofenac Potassium	10	62.0±0.01
M. pruriens	10	9.80±0.13
M. pruriens	50	47.8±0.01
Formalin (control)	0.2 ml	-
Diclofenac Potassium	10	46.8±0.21
M. pruriens	10	6.60±0.01
M. pruriens	50	38.8±0.18

Values are mean of triplicate determination ± standard error

Free radicals are molecules that have an unpaired electron, which creates the said molecule to become unstable. These free radicals can either accept an electron from or donate an electron to another molecule, thus, free radicals behaving as oxidants or reductants, respectively. Free radicals attack important molecules inside a cell, such as DNA, proteins, and lipids, and this may lead to the possible development of cancer. Free radicals in the body have been associated with a lot of degenerative

diseases such as Parkinson and Alzheimer diseases. They cause premature ageing of skin [22]. Antioxidants search for these free radicals and lend them an electron. This stabilizes the molecule, thus preventing damage to other cells. Antioxidants also turn free radicals into waste by products, and they eventually get eliminated from the body. They also have the ability to repair previous damage to cells.

Table 2. Antibacterial activity screening of the ethanol extracts of seeds of *M. pruriens* on the pathogens

Pathogens	*M. pruriens*
Proteus mirabilis	16.00
Klebsiella pneumonia	11.00
Staphylococus aureus	14.00
Pseudomonas aeruginosa	13.00
Escherichia coli	11.00

Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by scavenging free radicals produced in the body [23]. Consumption of these seeds may play a role in preventing human diseases in which free radicals are involved such as cancer, parkinson disease, cardiovascular disease and ageing. This may be the reason why the seeds of *M. pruriens* are used traditionally to cure parkinson disease and enhance libido in ageing people [24].

The effects of *M. pruriens* and diclofenac potassium on the proliferative phase of inflammation is summarized in Table 1 and the chats on Figs. 2 and 3. The anti-inflammatory activities were measured at the dose of 10 and 50 mg/kg against acute and chronic paw edema induced by carrageenan and formalin. The *M. pruriens* produced anti-inflammatory activity and the results were compared to that of diclofenac potassium as standard anti-inflammatory drug. Administration of *M. pruriens* at the doses 10 and 50 mg/kg showed an inhibition of 9.80 and 47.80% against acute paw edema induced by carragenaan; and inhibition of 6.60 and 38.80% against chronic paw edema induced by formalin.

In this study it was found that there was a reduction in the number of papules on the paw after the rats were fed orally with the seeds of *M. pruriens*. This showed that the plant materials have a significant activity on the test system at the dose levels tested.

Extracts of the seeds of *M. pruriens* exhibited strong antibacterial activities on the pathogens tested as shown in Table 2. The seeds extracts inhibited all the tested organisms *P. mirabilis, K. pneumonia*, *S. aureus*. *P. aeruginosa and E. coli. P. mirabilis and E. coli organisms* are the common cause of urinary tract infection and travellers diarrhea [23]. This finding indicates that the seeds can be used in the treatment of diarrhea and cholera. The seeds extracts of the plant showed inhibition against *K. pneumonia* and *S. aureus*. This implies that the seeds of *M. pruriens* may be useful in the treatment of wounds for which *S. aureus* is associated with [25]. The minimum inhibitory concentration (MIC) is shown in Table 3. The MIC of the seeds extracts is 12.5 mg/ml to 25.00 mg/ml. The ability of the seeds to inhibit the organisms thus suggests that the seeds would be useful in the treatment of infections and wounds.

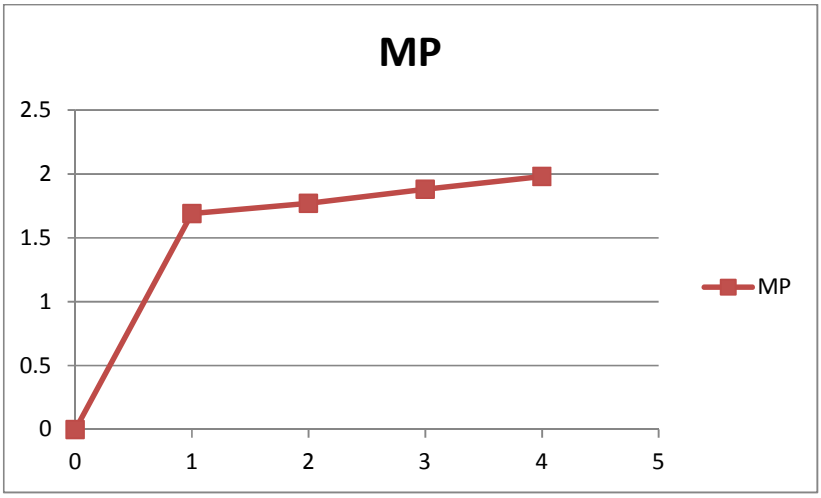

Fig. 1. Antioxidant activities of the seeds of *M. pruriens*

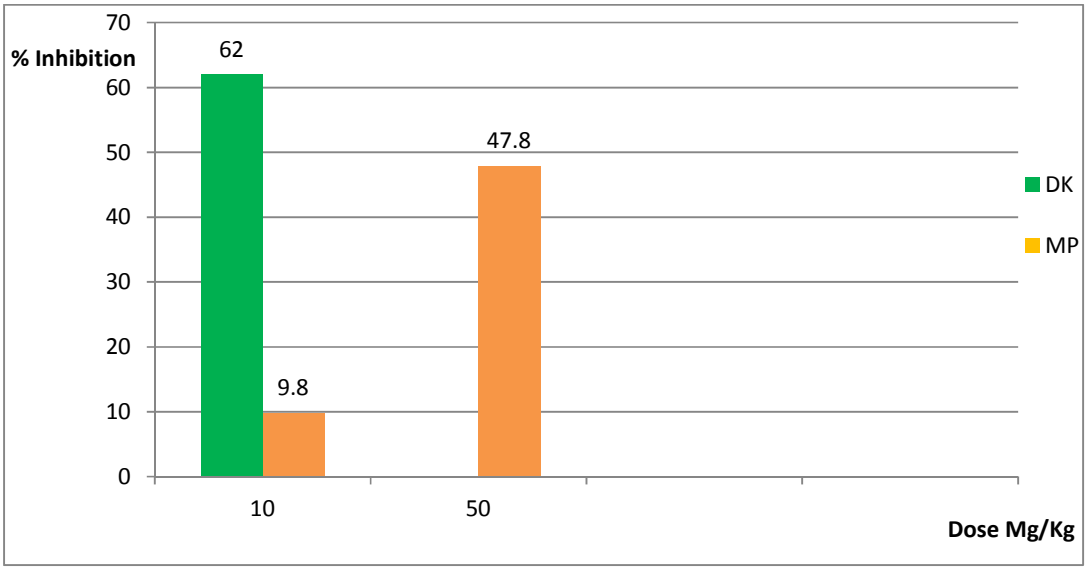

Fig. 2. Effect of the ethanol extracts of *M. pruriens* on acute paw edema

Where DK = Diclofenac Patassium
MP = M. pruriens

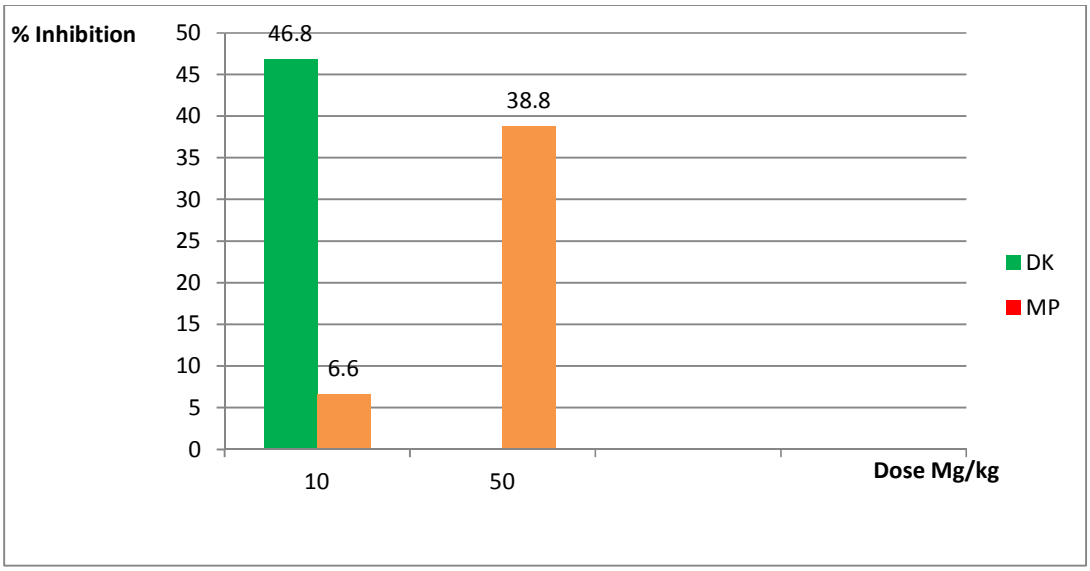

Fig. 3. Effect of the ethanol extracts of *M. pruriens* on chronic paw edema

Table 3. Minimum inhibitory concentration of the seeds of *M. pruriens* on the pathogens (mg/ml)

Pathogens	100	50	25	12.5	6.25	MIC
	zone of inhibition in mm					
Proteus mirabilis	16.00	11.00	5.00	2.00	-	12.5
Klebsiella pneumonia	11.00	7.00	3.00	-	-	25.00
Staphylococus aureus	14.00	12.00	8.00	5.00	-	12.5
Pseudomonas aeruginosa	13.00	10.00	6.00	2.00	-	12.5
Escherichia coli	11.00	8.00	4.00	2.00	-	12.5

Where DK = Diclofenac Patassium
MP = M. pruriens

4. CONCLUSION

The results of these analyses show that the seeds of *Mucuna pruriens* are safe and can be used as alternative therapeutic agents. As a result of the emergence of multi-drug resistant (MDR) bacteria, which is a major cause of treatment failure in many infectious diseases and which is becoming a worldwide public health concern [26], naturally occurring antimicrobials are being sought as replacements for synthetic ones. This analysis justifies the use of the seeds of *Mucuna pruriens* for the treatment fever, edema and elephantiasis etc. by the natives. It can be used as anti- inflammatory, antioxidant and antibacterial agent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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