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Evaluation of Phytochemical constituents, Total Phenolic contents and *in vitro* Antioxidant Activities of *Mucuna pruriens* fractions leaves

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Abstract

Mucuna pruriens is a tropical annual climbing legume plant used in traditional medicine systems to treat diabetes, arthritis, dysentery, hematinics, inflammation, and cardiovascular conditions. The present study investigates the phytochemical constituents, phenolic contents and *in vitro*, antioxidant potentials of *M. pruriens* leaf fractions using spectrophotometric methods. Phytochemical screening of hexane, chloroform, ethyl acetate, and methanol fractions of *M. pruriens* was determined using standard methods. The antioxidant activities of the fractions were assessed against total phenolic contents, total antioxidant contents, ferric ion reducing antioxidant power assay, and 1,1-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity using standard protocols. Phytochemical screening showed that only the ethyl acetate fraction of *M. pruriens* leaves contain all the phytochemical constituents tested for, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, phenol. The activities of the fractions against total phenolic contents were high in ethyl acetate fraction (1.958±0.24 mg GAE/g), while chloroform fraction gave the lowest total phenolic contents (1.476±0.19 mg GAE/g). On the contrary, chloroform fraction showed more significant total antioxidant contents (2.078±0.80 mg ASCE/g). The methanol fraction demonstrated higher ferric ion-reducing antioxidant power (4.866±1.19 mg ASCE/g). The methanol fraction scavenging activity against DPPH radical exhibited a lower IC_{50} value of 404.92 (µg/mL) relative to other fractions. It is concluded that ethyl acetate and methanolic fractions of *Mucuna pruriens* leaves, which contain large amounts of phenolic compounds, exhibited high antioxidant activities. These *in vitro* assays indicated that *Mucuna pruriens* leaves fraction is a significant source of natural antioxidants, which might help prevent the progress of various oxidative stresses.

Key words: *Mucuna pruriens,* Phytochemical, Fractions, Antioxidant, Oxidative stress, Free radicals,

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1.0 Introduction

The primary sources of traditional medicines against many illnesses for many years have been medicinal plants, microorganisms, and animals. Due to its accessibility, low cost, and lack of side effects, people living in underdeveloped nations rely heavily on natural products as a means of treatment [1]. About 80% of the world's population is thought to rely on traditional medicine for their primary healthcare, and the usage of herbal medicines continues to play a crucial role in primary healthcare [2]. They have attracted much interest due to their long history in folk medicine, and their positive effects on human health have attracted much interest in them, particularly in developing nations [3].

The remedies employed in different indigenous herbal practices to treat various diseases since very early times contain a significant amount of substances produced by plants. It is now well-established that medications made from plant products are safer than their synthetic counterparts and are the primary source of new medications used for treating and preventing diseases [4]. The significant antioxidant, antimutagenic, and anticarcinogenic potential of medicinal plants is related to their notable preventative and protective qualities [5].

Cancer, diabetes mellitus, cardiovascular illnesses, neurodegenerative disorders, and inflammatory diseases are among the diseases for which oxidative stress is primarily responsible for advancement and progression [6]. Excessive free oxygen and nitrogen production or unsuccessful quenching of those species within the cell leads to oxidative stress. Both exogenous (found in the environment) and endogenous (produced by the body) free oxygen and nitrogen species are unstable molecules that are involved in the body's normal aerobic

metabolic processes [7]. Cigarette smoke, ozone exposure, pharmaceuticals, ionizing radiation like X-rays, and other exogenous sources are examples of sources of free radicals.

On the other hand, endogenous sources of free radicals include mitochondrial electron transfer chain reaction processes, the xanthine oxidase pathway, and conditions associated with diseases such as inflammatory response, ischemia, and reperfusion injury [8]. The body possesses a strong antioxidant defense system made up of enzymatic and non-enzymatic pathways that, under normal physiological conditions, maintain a stable balance between pro-oxidants and antioxidants to ensure good health [6]. Superoxide dismutase, glutathione peroxidase, and catalase are enzymatic antioxidants. In contrast, the body uses non-enzymatic antioxidants such as lactoferrin, bilirubin, and uric acid, among others. However, in disease situations, the endogenous antioxidant systems become overburdened, causing an accumulation of excessive free radicals that induce oxidative stress-related damage to cellular machinery, which is linked to a number of diseases [9].

An antioxidant reduces the risk of acquiring degenerative diseases by reducing or delaying oxidative damage to organisms' cells caused by free radicals like peroxide or hydroperoxide [10]. There are primarily two approaches that have been suggested for this, one is to utilize antioxidants that directly scavenge free radicals, and the other is to find antioxidants that would boost the production of antioxidant enzymes [11]. Epidemiological and *in vitro* research shows that plant-based foods with antioxidant phytochemicals protect against various diseases. Butylated hydroxyl toluene, butylated hydroxyl anisole, and tetra butyl hydroquinone are examples of synthetic antioxidants that have been employed often. However, it has been claimed that these synthetic antioxidants are carcinogenic and have various modes of action [12]. These findings have motivated massive plant screening for potential medicinal and antioxidant activities, the isolation and characterization of various phytochemicals, and the use of antioxidants of natural origin to prevent illnesses [12].

Polyphenols and vitamins (A, C and E) are the two main classes of phytochemicals that

contribute to plants' ability to act as antioxidants. Plants produce phenolic chemicals, which are hydroxylated derivatives of benzoic and cinnamic acids and have anti-inflammatory and anticancer properties [13]. They contain tannins, anthocyanidins, coumarins, flavonoids, phenols, and flavonoids. The plant defense systems against biotic and abiotic stressors depend on these phytoactive compounds [14]. It is believed that consuming plants or products high in these phytoactive principles will have the same positive benefits on humans [15]. For instance, anti-inflammatory, anti-allergic, antiviral, immunomodulatory, anti-aging, and antiproliferative effects of flavonoids have long been known [16]. Research on dietary and medicinal plants that can prevent, treat, or mitigate diseases brought on by oxidative stress has received much attention due to the search for superior antioxidants to synthetic ones [13, 16].

Numerous studies have evaluated the antioxidant potential of medicinal plants like *Mucuna pruriens* [17]. *Mucuna pruriens* is a tropical annual climbing legume belonging to the Fabaceae family [18]. *M. pruriens,* also known as the velvet bean, is a plant with a height range of 3 to 18 meters. The plant can be found most frequently in the West Indies, India, and Africa. Its names in Nigerian include agbala or akurugba, werepe or yerepe, and karara, in Igbo, Yoruba and Hausa respectively [19]. It covers most of the subcontinent, and soft, dry-deciduous woods, bushes, and hedges blanket the plains of India. The thick, hairy, leathery, and four to six-seed pods resemble violin sound holes. They have thick, luxurious, and dark brown hair. *M. pruriens* leaves are highly regarded in South-Eastern Nigeria as superior natural blood boosters, especially for incapacitating conditions, unexpected blood loss, and anemia [19]. Numerous illnesses, such as diabetes, arthritis, dysentery, and cardiovascular conditions, are treated with *M. pruriens* leaves. Researchers have discovered that the seeds of *M. pruriens* have trypsin inhibitory, hepatoprotective, and antioxidant properties [20].

Mucuna pruriens contain a variety of alkaloids and flavonoids, which include bufotenin, dimethyltryptamine, tetrahydroquinolone alkaloids, stizolamine, mucunine, mucunadine, prurienidine,

nicotine, genistein, medicarpin, kievitone, and cajanol, among others [21-22]. In spite of the fact that, the phytochemical constituents, antioxidants and pharmacological activities of different extracts of *Mucuna pruriens* had been previously investigated. However, there is very little information on the effects of solvent polarity on phytochemical constituents and antioxidants activities of *Mucuna* pruriens leaves found in south west, Nigeria. Therefore, the current study evaluated phytochemical constituents, total phenolic contents and *in vitro* antioxidant activities of *Mucuna pruriens* fractions leaves.

2.0 Materials and Methods

2.1 Collection of plant materials

The leaves of *Mucuna pruriens* were obtained from a popular herbs market, 'Oje,' Ibadan, Oyo/State, Nigeria. The plant sample was identified at the herbarium of the Forestry Research Institute of Nigeria (FRIN). A voucher specimen with the number F.H.I. 112974 was deposited at the FRIN herbarium. The plant was authenticated at FRIN.

2.2 Preparation of crude extract

The fresh leaves of *Mucuna pruriens* were dried and ground into powder at room temperature. Three hundred grams (300 g) of powdered plant material was weighed and extracted with 1.5 L of mixture of methanol and water (80:20) for 72h at about 64^{o}C using the Soxhlet extractor apparatus. After filtration, the extract *of Mucuna pruriens* was concentrated under low pressure and 40^{o}C temperature on a rotary evaporator. The crude extract of *Mucuna pruriens* was extracted in a separating funnel using a liquid-liquid fractionation process. This was partitioned separately using 1000 mL each of hexane, chloroform, ethyl acetate, and methanol solvents. Following concentration on a rotary evaporator with reduced pressure, the various fractions of hexane, chloroform, ethyl acetate, and methanol of *Mucuna pruriens* were concentrated to dryness and stored at -20^{o}C.

2.3 Phytochemical analysis of the crude extract

Phytochemical screening of hexane, chloroform, ethylacetate, and methanol fractions of *Mucuna pruriens* leaves was done as previously described by Ekwueme *et al*. [23]. The presence of selected

phytochemical constituents such as saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenol was investigated.

2.4 Determination of antioxidant potential of *Mucuna pruriens* fractions

The total phenolic contents (TPC), ferric ion reducing antioxidant power assay (FRAP), total antioxidant content (TAC), and 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical-scavenging activity hexane, chloroform, ethylacetate, and methanol fractions of *Mucuna pruriens* leaves were evaluated.

Total phenolic contents (TPC)

The total phenolic contents were determined spectrophotometrically by the Folin-Ciocalteu assay. This assay is based on the Ebrahimzadeh method [24[. This is a colorimetric oxidation/reduction method for phenolic compounds. The hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* leaves were diluted to different concentrations, 200, 400, 600, 800, and 1000 µg/mL, and 0.5 ml of different dilutions were separately mixed, each with 5mL of Folin-Ciocalteu reagent (to get a ratio of 1:10 dilution) and allowed to stand for 5 min before neutralized with 4mL 1M aqueous sodium carbonate. The resulting solution was allowed to stand for another 15 min, and the phenols were determined by colorimetric method at 765 nm. The concentrations of phenolic compounds in the fractions were expressed as mg of gallic acid equivalents (GAEs) per gram of the dry fraction.

Total antioxidant contents (TAC)

The total antioxidant capacity of the hexane, chloroform, ethylacetate and methanol fractions of *Mucuna pruriens* was evaluated by the phosphomolybdenum method according to the procedure previously described by Phatak and Hendre [25]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. The molybdate reagent solution was prepared by adding 20 mL of distilled water with 1mL of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate and made up to 50 mL with distilled water.

The hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* were

serially diluted to five different concentrations (200, 400, 600, 800, and 1000 µg/mL) and equal volumes added to each of the five test tubes individually containing 3 mL of distilled water and 1 mL of molybdate reagent solution. These test tubes with reaction solution were incubated at 95^0C for 90 min after which the tubes were incubated at room temperature for 20-30 min, and then absorbance of the reaction solution was measured at 695 nm. The antioxidant activity results were expressed as mg ascorbic acid equivalent (ASCE) per gram of dry fraction.

Ferric ion reducing antioxidant power assay (FRAP)

The antioxidant activity was determined based on the ability of antioxidants in the hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* to reduce ferric to ferrous in FRAP reagent [25]. The FRAP reagent was prepared by diluting the fractions to different concentrations (200, 400, 600, 800, and 1000 µg/mL) and later mixed each concentration with 2.5 ml of 20 mM phosphate buffer and 2.5 mL 1%, w/v potassium ferric cyanide. The mixtures were later incubated at 50°C for 30 min, and afterward, 2.5 mL of 10%, w/v trichloroacetic acid and 0.5 mL of 0.1%, w/v ferric chloride were added to the mixtures and kept for 10 min. Ascorbic acid was used as a standard reference control, and the absorbance was measured at 700 nm. All samples were measured in triplicate, and the mean values were recorded. The results were expressed as mg ascorbic acid equivalent (ASCE) per gram of dry fraction.

DPPH free radical-scavenging activity evaluation

The free radical-scavenging activity of the hexane, chloroform, ethyl acetate, and methanol fractions *of Mucuna pruriens* leaves were determined against 1,1-diphenyl-2-picryl hydrazyl radical as previously described by Ebrahimzadeh *et al.* [24]. The fractions were diluted to different concentrations (200, 400, 600, 800, and 1000 µg/mL). After that, equal volumes were added to the methanolic solution of DPPH (100 µM). The mixture was left for 15 mins at room temperature. The absorbance was measured at 517 nm. The procedure was done in triplicate, and the mean values were recorded. Ascorbic acid

(Vitamin C) was used as standard antioxidant control at the same concentrations and measured at the same wavelength with the fractions. The percentage of inhibition was evaluated using the equation below:

% Inhibition = (ABcont – ABsamp)/ABcont × 100

cont = control

samp= sample

Where % Inhibition = percentage of inhibition; $AB_{control}$ = Absorbance of control; ABsample = Absorbance of test sample.

The IC_{50} value is the concentration of sample required for scavenging 50% of the radicals.

2.5 Statistical analysis

The mean and Standard Error of Mean (SEM) of the data was done using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. The *P* value < 0.05 was considered significant. The IC_{50} values were determined by linear regression analysis.

3.0 Results

3.1 Phytochemical screening

The results of the phytochemical screening of hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* leaves are given in Table 1. The hexane fraction showed the presence of anthraquinones, terpenoids, and steroids. The chloroform fraction gave the presence of saponins, tannins, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenol. In addition, the ethylacetate fraction confirmed the presence of all the phytochemicals screened for, saponins, tannins, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, phenol, and flavonoids. Methanol fraction showed the presence of saponins, tannins, anthraquinones, terpenoids, steroids, alkaloids, phenol, and flavonoids.

Table 1: Preliminary phytochemical screening of the n-hexane, dichloromethane, ethylacetate, and aqueous fractions *Mucuna pruriens* leaves

Phytochemical Constituents	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
Saponins	-ve	+ve	+ve	++ve
Tannins	-ve	+ve	+ve	++ve
Flavonoids	-ve	-ve	+ve	++ve

Cardiac glycosides	-ve	+ve	+ve	-ve
Anthraquinones	+ve	+ve	+ve	+ve
Terpenoids	++ve	++ve	+ve	+ve
Steroids	+ve	+ve	+ve	++ve
Alkaloids	-ve	+ve	++ve	++ve
Phenol	-ve	+ve	+ve	+ve

INTERPRETATIONS

+ve : Present

++ve : Abundantly present

-ve : Absent

3.2 Antioxidant results of *Mucuna pruriens* fractions

Total Phenolic Contents

Total Phenolic Contents of hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* leaves were expressed as gallic acid equivalents mg (GAE) per gram of dry fraction. The highest phenolic content was found in ethlyacetate fraction (1.958±0.24 mg of GAE/g), followed by methanol (1.736±0.33 mg of GAE/g), hexane (1.556±0.27 mg of GAE/g) and chloroform (1.476±0.19 mg of GAE/g) (Table 2).

Table 2: Total Phenolic Contents (TPC) in different fractions of *Mucuna pruriens* leaves

S/No.	Fractions	200 (µg/mL)	400 (µg/mL)	600 (µg/mL)	800 (µg/mL)	1000 (µg/mL)	Mean TPC (GAE/g)
1.	Hexane	1.240	1.357	1.590	1.675	1.917	1.556±0.27

2.	Chloroform	1.270	1.333	1.429	1.619	1.731	1.476±0.19
3.	Ethyl acetate	1.588	1.868	2.048	2.124	2.160	1.958±0.24
4.	Methanol	1.317	1.556	1.699	1.951	2.158	1.736±0.33

The Mean TPC value in the table is expressed as the Mean ± SEM

Total antioxidant contents (TAC)

All the fractions showed different degrees of antioxidant activity, as shown in Table 3. All fractions showed increasing antioxidant activity with increasing concentration. However, it was shown that the chloroform fraction of *M. pruriens* possesses the highest total antioxidant contents of 2.078±0.80 mg/g ascorbic acid equivalent, followed by ethyl acetate (1.746±0.56 mg of ASCE/g), hexane (1.36±0.205 mg of ASCE/g) and methanol (1.040±0.11 mg of ASCE/g).

Table 3: Total Antioxidant Contents in different fractions of *Mucuna pruriens* leaves

S/No.	Fractions	200 (µg/mL)	400 (µg/mL)	600 (µg/mL)	800 (µg/mL)	1000 (µg/mL)	Mean TAC (ASCE/g)
1.	Hexane	1.030	1.380	1.390	1.490	1.600	1.36±0.205
2.	Chloroform	1.200	1.550	1.840	2.650	3.150	2.078±0.80

3.	Ethyl acetate	1.040	1.500	1.640	2.080	2.480	1.746±0.56
4.	Methanol	0.980	0.980	0.980	1.020	1.240	1.040±0.11

The Mean TAC value in the table is expressed as the Mean ± SEM

Ferric Reducing Antioxidant Power (FRAP)

Generally, all the fractions of *Mucuna pruriens* leaves exhibited remarkable concentration-dependent increases in reducing power (Table 4). The methanol fraction of *M. pruriens* showed high reducing power of 4.866±1.19 mg of ASCE/g, followed by chloroform (3.416±1.45 mg of ASCE/g), ethyl acetate (3.407±1.03 mg of ASCE/g) and hexane (2.951±0.51 mg of ASCE/g)

Table 4: Ferric ion reducing antioxidant power assay in different fractions of *Mucuna pruriens* leaves

S/No.	Fractions	200 (µg/mL)	400 (µg/mL)	600 (µg/mL)	800 (µg/mL)	1000 (µg/mL)	Mean FRAP (ASCE/g)
1.	Hexane	2.059	3.042	3.126	3.147	3.383	2.951±0.51
2.	Chloroform	2.206	2. 392	2.516	3.474	5.490	3.468±1.43
3.	Ethyl acetate	2.017	2.726	3.740	3.966	4.586	3.407±1.03

4.	Methanol	2.889	4.744	5.275	5.401	6.021
4.866±1.19						

The Mean FRAP value in the table is expressed as the Mean ± SEM

Free radical scavenging activity by the DPPH method

The results of the DPPH radical scavenging activity of the hexane, chloroform, ethyl acetate, and methanol fractions of *Mucuna pruriens* leaves and the standard ascorbic acid are presented in Table 5. All the fractions of *Mucuna pruriens* leave tested demonstrated notable *in vitro* DPPH radical scavenging activities in a dose-dependent trend. The standard (ascorbic acid) showed higher DPPH radical scavenging activities than all the fractions of *Mucuna pruriens* leaves tested (Table 5). The concentrations of the studied fractions required to scavenge 50% of the DPPH radicals (IC_{50}) were also determined in this study. The order of reducing IC_{50} values which is an indication of increasing free radical scavenging activity are as follows, methanol (404.92 µg/mL), ethyl acetate (518.44 µg/mL), hexane (574.68 µg/mL) and chloroform (1088.67 µg/mL) fractions On the other hand, the IC_{50} value of the standard (ascorbic acid) was180.62 µg/mL (Table 5 and Figure 1).

Table 5: *In vitro* DPPH scavenging activities of different fractions of *Mucuna pruriens* leaves and Ascorbic acid at different concentrations

	DPPH scavenging activity (% inhibition)			
Concentrations (µg/mL)	**Ascorbic acid**	**Hexane fraction**	**Chloroform fraction**	**Ethyl acetate Methanol fractiom**
1000	96.99	57.42	30.41	64.04
83.11				

800	95.82	48.24	26.12	61.36
83.02				
600	95.68	38.70	17.17	57.78
82.65				
400	95.68	24.49	10.38	54.03
73.65				
200	95.60	11.60	2.33	46.33
37.48				
100	95.53	4.21	0.07	42.15
13.35				
0	0.00	0.00	0.00	0.00
0.00				
IC_{50} (µg/mL)	180.62	574.68	1088.67	518.44
404.92				

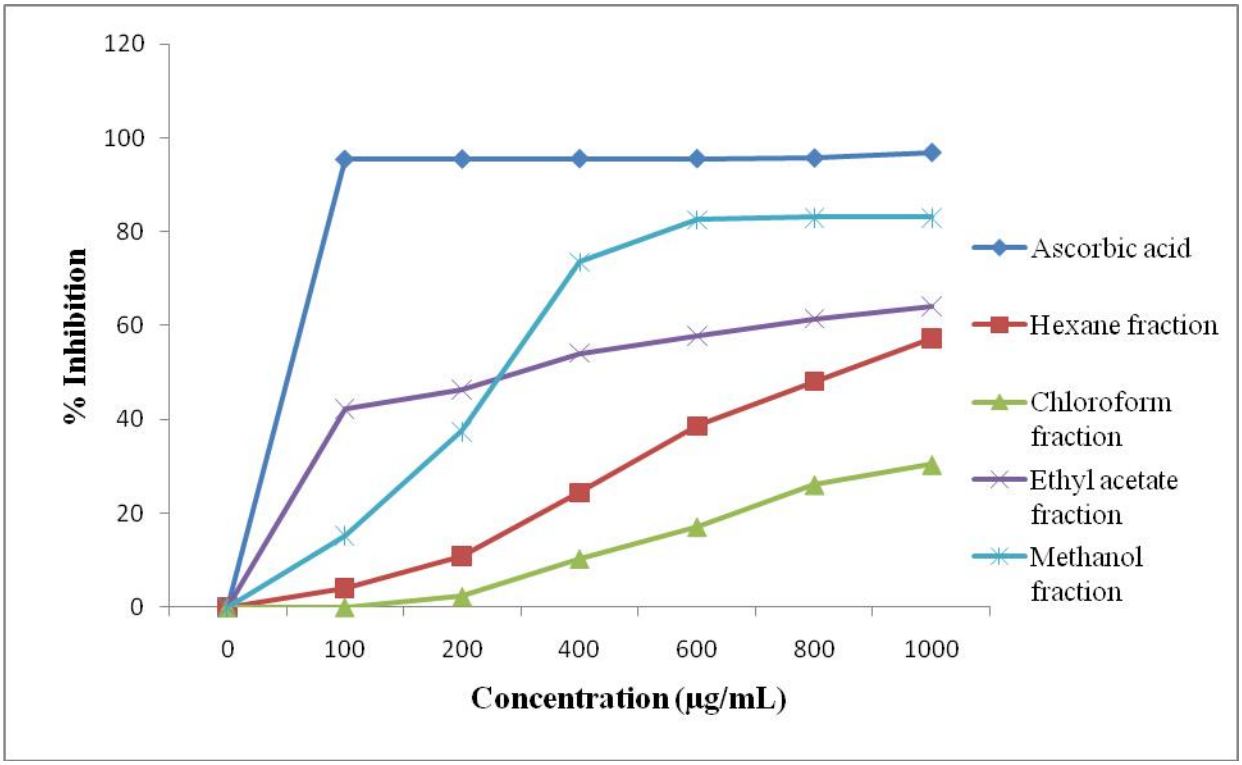

Figure 1: Plot of DPPH radical scavenging percentage between ascorbic acid and hexane, dichloromethane, ethyl acetate and methanol fractions of *Mucuna pruriens* leaves

4.0 Discussion

The medicinal benefits of plant leaves may be due to the phytochemicals that make up those leaves. In the four fractions of *Mucuna prurien* leaves, the phytochemical screening revealed anthraquinones, terpenoids, and steroids in all the fractions, while saponins, tannins, cardiac glycosides, and alkaloids were detected in chloroform, ethylacetate and methanol fractions alone. Only the ethylacetate and methanol fractions contained flavonoids. The secondary metabolites (phytochemicals) and other chemical components of medicinal plants account for their medicinal value, according to Varadarajan *et al.* [26]. The presence of phenolic compounds in *Mucuna pruriens* leaves is responsible for its' antioxidative characteristics and their value as herbal remedies [27]. The good antioxidative activity of the ethyl acetate and methanol fractions of *Mucuna pruriens* leaves in our current study may be explained by the fact that flavonoids have also been shown to

exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes like the ATPase and phospholipase A2 [27].

In the current study, the ethyl acetate and methanol fractions of *Mucuna pruriens* leaves contained abundant alkaloids, which may be responsible for their high antioxidant property, which has been well established by Anosike *et al*. [31], using methanol extract of *Mucuna pruriens* leaves. Additionally, the presence of saponins in the chloroform, ethyl acetate, and methanol fractions, supports earlier research by Elekofehinti *et al*. [28], which demonstrated the antioxidative and antiperoxidative characteristics of saponins. However, a prior investigation found that plants' hypotensive and cardiodepressant effects were caused by the presence of steroids and cardiac glycosides [29]. Furthermore, anthraquinones has a bactericidal, astringent, purgative, anti-inflammatory, mild anticancer and anti-inflammatory effect [30].

Research has indicated that antioxidant activities ought not to be established based on a single antioxidant experimental model. Several *in vitro* examination techniques are considered for assessing antioxidant activities [32]. The antioxidant capacity of the plant extract is therefore determined by the quenching, scavenging, or suppression of the production of free radicals. The primary antioxidant phytochemicals are phenolic substances such as tannins, flavonoids, and phenolic acids [33]. This property results from their ability to produce reducing oxides, which are crucial for the adsorption or neutralization of free radicals [34].

The ethyl acetate fraction contained flavonoids and tannins, demonstrated the highest total phenolic contents of 1.958±0.24 mg of GAE/g. However, the absence of flavonoids in chloroform fraction may have been responsible for the minimal value of total phenolic contents of 1.476±0.19 mg of GAE/g in our study. Our current finding is consistent with Theansungnoen *et al*. [35], who linked total phenolic contents with solvent polarity using seed extracts of four Mucuna Species. Therefore, this work showed a correlation between the hydrophilic nature of phenolic chemicals and the number of phytoconstituents extracted (Table 2).

Reactive oxygen species are quenched by the redox characteristics of phytochemicals such as phenolic compounds, which are crucial for the absorption and neutralization of free radicals, the breakdown of peroxides, and the neutralization of oxidative stress [37]. Total antioxidant contents are determined using the phosphomolybdenum test, a quantitative method for evaluating antioxidant capabilities [36]. The result showed increasing antioxidant activity with increasing concentration. However, it was found that the chloroform fraction of *M. pruriens* leaves exhibited the highest total antioxidant contents (2.078±0.80mg of ASCE/g), followed by the ethyl acetate fraction (1.7460.56 mg of ASCE/g). A lower level of antioxidant activity was found in the methanol fraction of *M. pruriens* leaves (1.040.00.11 mg of ASCE/g). The results exhibited by chloroform and methanol fractions are not in total agreement with previous findings by Kottai Muthu *et al.* [17], who linked high total antioxidant contents with the presence of flavonoids, using *in vitro* antioxidant activity of various extracts of *Mucuna pruriens* whole plant.

The reducing capacity of a compound may provide important information about its antioxidant activity [38]. Earlier researchers, Tanaka *et al*., [39], have experimented with a direct relationship between antioxidant activity and the reducing power of some plant extracts. Reductones are typically linked to the presence of reducing properties [40]. Reductones have been shown to have antioxidant effects by breaking the chain of free radicals by donating a hydrogen atom [41]. According to reports, reductones interact with specific peroxide precursors to stop the creation of peroxide. Table 4 shows the reductive capabilities of the plant fractions compared to the ascorbic acid equivalent. In contrast to other fractions studied, a notable reducing capacity was discovered in the methanol fraction of *Mucuna pruriens* leaves. The activity was found in the order methanol fraction > chloroform fraction > ethyl acetate fraction > hexane fraction. The Ferric ion-reducing antioxidant power results are inconsistent with Elkhamlichia *et al.* [42] findings, which relate the reducing power activities of seeds and pods extracts of *Calycotome villos* with phenolic contents. As the concentration of the fractions

gradually increased, it was seen that the reducing power of the fractions rose. These findings imply that phenols or other chemicals that can donate hydrogen are present in all the fractions [42].

DPPH (1,1 diphenyl-2-picrylhydrazil) is a stable free radical widely used to assess the radical-scavenging capacity of natural substances. Antioxidants convert DPPH to DPPH-H by a reaction, which lowers absorbance. The discoloration level reveals the antioxidant compounds' scavenging capacity in terms of their capacity to donate hydrogen [43]. Antioxidants found in the leaves of *Mucuna pruriens* can scavenge the DPPH radical by donating a proton to create the reduced DPPH. In this study, the DPPH radical scavenging activity of the *M. pruriens* leaves fractions was concentration dependent, contrary to the previous report on the DPPH radical scavenging activity of methanol extract of *Mucuna pruriens* leaves by Anosike *et al*. [31]. The antioxidant activity of the fractions was expressed as $IC_{50,}$ which indicates the fractions' inhibitory concentration (µg/mL) that inhibited the formation of DPPH radicals by 50%. Ascorbic acid was used as a positive control. In research under investigation, the IC_{50} for the standard, methanol, ethyl acetate, hexane, and chloroform fractions were 180.62, 404.92, 518.44, 574.68, and1088.67µg/mL, respectively. Meanwhile, our findings provided better IC_{50} values when compared with the whole plant of Petroleum ether and methanol extract of *Mucuna pruriens* (1030 µg/mL and 1230 µg/mL), respectively [44]. The current work confirms previous research by Khan *et al*. [44], who asserted that flavonoids and related polyphenols play a substantial role in the antioxidant activity of medicinal plants. This is caused by the presence of hydroxyl groups and conjugated ring structures. Furthermore, many phenolic compounds can act as antioxidants by neutralizing or stabilizing free radicals implicated in oxidative processes through hydrogenation or by complexing with oxidizing species [45].

5.0 Conclusion

The identified phytochemicals make the *Mucuna pruriens* leaves fractions pharmacologically active. Their antioxidant activity may be responsible for their usefulness in managing and treating various diseases. We are studying other possible

mechanisms of action of these leaf fractions. Efforts to identify the constituent compounds responsible for this antioxidant activity are also in progress.

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