Plant Biotechnology Persa
Ilam University of Medical Sciences
Online ISSN: 2676-7414
Homepage: https://pbp.medilam.ac.ir

Bioactive compound identification and phytochemical analysis of *Mucuna pruriens* seed extract

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Article Info

Article type:
Original Article

Article History:
Received: 21 July 2025
Revised: 22 Oct 2025
Accepted: 23 Oct 2025
Published Online: 30 Oct 2025

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A B S T R A C T

Objective: *Mucuna pruriens*, commonly known as velvet bean, is a climbing legume belonging to the family Fabaceae. The aim of the present study was to investigate qualitative and quantitative phytochemical analysis and to identify the bioactive compounds in the ethanolic extract of *M. pruriens* seed using GC-MS technique.

Methods: Dried powder form of Mucuna pruriens was extracted with ethanol by using a 1:10 dilution, in cold maceration. The extract was evaporated into dryness using a hot water bath for 72 hrs. The GC-MS analysis of M. pruriens seed extract was performed using GC -MS equipment (Agilent Technologies) to identify the phytochemical constituents. While the compound mass spectra contained in the extract has been matched with the National Institute of Standards and Technology (NIST) library.

Result: The preliminary phytochemical screening revealed the presence of Alkaloids, flavonoids, phenol, saponin, steroids, tannin and terpenoids. The analysis by Gas chromatography-mass spectrometry results showed the presence of five major bioactive compounds.

Conclusion: Antioxidant, antifungal, antimicrobial, anti-inflammatory, anti-cancer, and anti-Parkinsonian properties have been reported to compounds which were found in present study.

Keywords: *Mucuna pruriens*; phytochemical constituents; GC-MS; bioactive compounds; antioxidant activity

➢ **How to cite this paper**
Germame Y, Atnafu H. Bioactive compound identification and phytochemical analysis of *Mucuna pruriens* seed extract. Plant Biotechnology Persa. 2026; 8(2): Proof.

Introduction

Mucuna pruriens is an underutilized medicinal plant that belongs to the family Fabaceae (Leguminosae), which are a large group of flowering plants with a world-wide distribution. Many are economically or ecologically important. The family consists of about 18,000 species in 630 genera [1].

Mucuna seeds are great source of L-Dopa a non-protein phenolic amino acid and precursor of the brain neurotransmitter dopamine [2]. In addition to L-Dopa, pharmacologically active compounds methylated and non-methylated tetra hydroisoquinoline are also present in the seed of mucuna [3]. The seed also contains high concentration of bioactive compounds such as free phenolics, tannins, phytic acid and other nutrients [4].

Plants are known by their secondary metabolites responsible for pharmacological activities. The majority of these phytochemicals are antioxidants [5]. Humans have been utilized medicinal plants as herbal medicine in the treatment of various diseases [6]. It has been reported that many plants actually have medicinal value [7]. Every part of *M. pruriens* possess valuable medicinal properties and it has been explored in various contexts, including for its anti-diabetic, aphrodisiac, anti-neoplastic, anti-epileptic, anti-ageing, rheumatoid arthritis and

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[DOI: 10.66224/pbp.8.3.2]

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anti-microbial activities [8,9]. Its anti-venom property has been investigated by [10], anti-helminthic activity has been reported by [11]. *M. pruriens* has also been exhibited to be neuroprotective [12], and has showed analgesic and anti-inflammatory activity [13].

In Ethiopia, this valuable medicinal plant is not nationally known for its use. Rather, it has only been known and traditionally used by the local communities around Gambella region. One of the major reasons to this is lack of prior scientific studies that characterize its chemical constitutes and potential health benefits of this valuable species in the country. In fact, some scientific study has been carried out on the phytoconstituents of the seeds based on its geographical distribution in some other countries. However, phytoconstituent profile of plants are subject to significant variations related to geographical location, climatic condition, harvest, processing technique and some other conditions [14]. Therefore, this study aimed to perform qualitative and quantitative phytochemical analyses and identify bioactive compounds in the seeds of *M. pruriens* to support their potential valorization.

Material and methods

Sample collection and identification

Mature seeds of *M. pruriens* were collected from Gambella town in the southwestern Ethiopia. The plant was identified and authenticated by a botanist, at Ethiopian biodiversity institute.

Sample preparation

The collected seeds were shade dried for 15 days and ground into fine powder, which was then sieved and stored in an air tight bag for further analysis.

Solvent extraction

The dried powder form of *M. pruriens* was extracted by using a 1:10 dilution in ethanol, in cold maceration. For this,10 g of seed powder was soaked in 100 ml of organic solvent (ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 48 hrs with a humidity condition of 80-90%. The extract was filtered using a Whatmann filter paper No. 42 (125mm). The sample was subsequently concentrated using a rotating evaporator at a temperature of 40°C under reduced pressure. Finally, the concentrated extract was weighed and stored at a temperature of 4°C for further use.

Qualitative phytochemical analysis

Phytochemical screening

The Phytochemical analysis of the extract was conducted to determine the presence of active secondary metabolites. The phytochemical investigation based on ethno-pharmacological information is generally considered as successful approach in the discovery of new anti-infective agents from higher plants [15]. The plant extract was screened for the presence of reducing sugars, alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, terpenoids and cardiac glycosides according to establish procedures. [16, 17,18,19].

Alkaloids: 20 mL of sample extract was evaporated to dryness in an evaporating dish on water bath. 5 mL of 2N HCL was added and stirred while heating on a water-bath for 10 minutes cooled and filtered. A drop of Mayer's reagent was added to the filtrate. The sample was then observed for the presence of turbidity or precipitation [17].

Saponins: 2 mL of sample was heated in 10 mL of distilled water and shaken vigorously. The formation of a persistent froth was taken as an indication for the presence of saponins [18].

Flavonoids: A portion of aqueous filtrate of extract was mixed with 5 mL of dilute ammonia solution. Then, concentrated H2SO4 was added to it drop by drop. A yellow coloration was taken as an indication for the presence of flavonoids [18].

Tannins: A drop of 0.1% ferric chloride was added to the 1g of powdered sample. Then the mixture was heated in a 20 mL of distilled water on a water bath. The mixture was filtered. The appearance of a brownish green or blue-black color was taken as an indication for the presence of tannins [16].

Terpenoids

Salkowski test: 2 mL of chloroform was added to the extract. Then 2 mL of concentrated H_2SO_4 was added and gently shaken. An appearance of reddish-brown color was taken as an indication for the presence of terpenoids [19].

Reducing sugars: 1 mL of extract was added to 1.5 mL of water and few drops of Fehling reagent was added and formation of brick red precipitate was taken as an indication for the presence of reducing sugars [17].

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Steroids: 2 mL of H_2SO_4 was added to 0.5 g of aqueous extract of sample. Then 2 mL of acetic anhydride was added to the solution. A color change from violet to blue was taken as an indication for the presence of steroids [18].

Quantitative phytochemical analysis

After qualitative phytochemical estimation, quantitative phytochemical estimation was done for the total phenolic and flavonoid count as the following:

Estimation of total phenol content determination

The total phenolic count of the extract was determined using the Folin-Ciocalteu method [17]. 1.8 mL fresh Folin was added to 1 mL of extract. After 5 min, 1.2 mL of sodium carbonate solution was added. Then the solution was stand for 90 min at 30^0C. The absorbance was measured at 765nm. Results were expressed as gallic acid equivalents per gram of sample.

Estimation of total Flavonoid content

The total flavonoids content was measured by a colorimetric assay [20]. 0.5 mL of extract and 2.4 mL of distilled water and After 5 min 0.3 mL of 5% NaNO2, 0.3 mL of 10% AlCl3 was added to the solution. Then after 6 min of incubation at room temperature, 1 mL of NaOH solution (5%) was added to the reaction mixture. Then the mixture was thoroughly mixed and allowed to stand for 15 min. Quercetin was used as a standard for the calibration curve. The absorbance was measured at 510 nm. Total flavonoid content of the extract was expressed as quercetin equivalents (QE) per gram of sample.

Estimation of total tannins content

1 mL of the sample extract was mixed with 0.5 mL of folin denis reagent followed by the addition of 1 mL of sodium carbonate and 8 mL of distilled water to the final mixture. The absorbance was read at 725 nm. A blank was prepared with water. The tannins content of the extract was expressed as tannic acid equivalents (TAE) per gram of sample [21].

GC-MS Analysis

Ethanolic extract was also analysed by GC-MS equipment. The temperature was set as, 70°C for 2 minutes, hold increased at 7°C/minute up to 200°C and then accelerated at 5°C/minute up to 220°C with 5 minutes hold. Injector temperature was set at 220°C. The scanning of mass range was from 35 to 400 (m/z). The control of the GC-MS system and data peak processing was controlled by means of Excalibur software. Phytoconstituents identification was verified based on the relative retention time and their peak area with the NIST and LIB database of the GC-MS system.

Identification of compounds

The GC-MS analysis identified compounds known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The identification of compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds available in the database of National Institute of Standard and Technology (NIST) and the name, molecular weight and structure determined.

Results

Qualitative phytochemical analysis

The phytochemical screening of the ethanolic seed extract of *M. pruriens* showed the presence of phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, steroids and reducing sugars as shown in Table 1.

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Table 1: qualitative phytochemical constituents of *Mucuna pruriens* seeds extract

S. No	Phytochemical constituent	*Mucuna pruriens* seeds extract
1	Alkaloids	++
2	Flavonoids	++
3	Tannin	++
4	Terpenoids	++
5	Saponins	++
6	Steroids	++
7	Reducing sugars	++

(++) = present.

Quantitative phytochemical analysis

The result from quantitative phytochemical analysis showed that highest amount of tannin followed by flavonoids and phenols as shown in Table 2.

Table 2: Quantitative phytochemical analysis of ethanolic seed extract of *mucuna pruriens*

Phytochemicals	**mg/g of extracts (GAE, QE, TAE)**
Total phenol	0.704±0.026
Total flavonoid	1.996±0.023
Total tannin	7.493±0.242

Statistical analysis

Data are expressed as mean ± standard deviation (SD) of triplicates.

Total Phenolic Content (TPC) expressed as (mg Gallic acid equivalent/ g extract)

Total Flavonoids contents (TFC) expressed as (mg Quercetin equivalents /g extract)

Total Tannin content (TTC) expressed as (mg tannic acid equivalents /g extract)

The amount of total tannin was determined using folin denis reagent. The calibration curve obtained for tannic acid is shown in figure 1. Tannic acid was used as a standard compound and the total tannin were expressed as mg/g gallic acid equivalent using the standard curve equation:

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y=0.1923x -0.0144, R2 = 0.9908 (Figure: 1) where y is absorbance at 725 nm and x is total tannin content in the extracts of *M. pruriens* expressed in mg/gm. The total tannin content was 7.493±0.242 mg/g in the seed extracts, shown in Table-2.

The amount of total flavonoid was determined with aluminum chloride reagent. The calibration curve for Quercetin is shown in figure2. Quercetin was used as a standard compound and the total flavonoid were expressed as mg/g Quercetin equivalent using the standard curve equation: y = 7.2065x+0.2286. R2 = 0.9969 (Figure 2). Where y is absorbance at 510 nm and x is total flavonoid content in the seed extracts *M. pruriens* expressed in mg/gm. The total flavonoid content was 1.996±0.023 mg/g in the seed extracts, (Table- 2).

The amount of total phenol was determined with Folin-Ciocalteu reagent. The calibration curve obtained for gallic acid is shown in figure 3. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 9.2859x+0.184, R2 = 0.9924 (Figure: 3) where y is absorbance at 765 nm and x is total phenolic content in the extracts of *M. pruriens* expressed in mg/gm. The total phenolic content was 0.704±0.026 mg/g in the seed extracts, shown in Table-2.

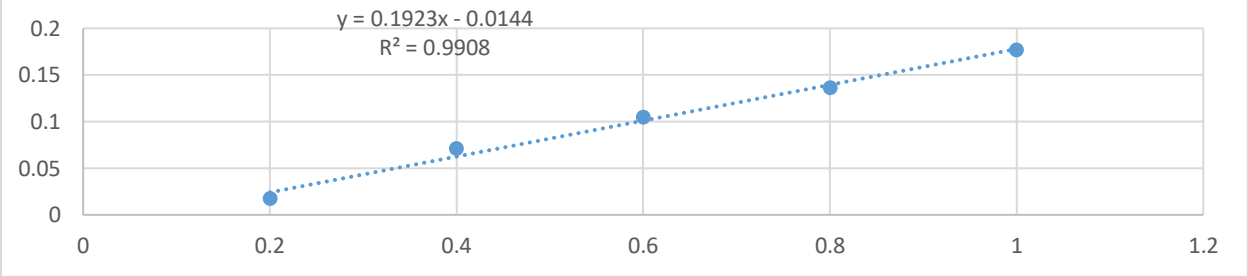


Fig 2: Standard calibration curve for total tannin content for standard tannic acid

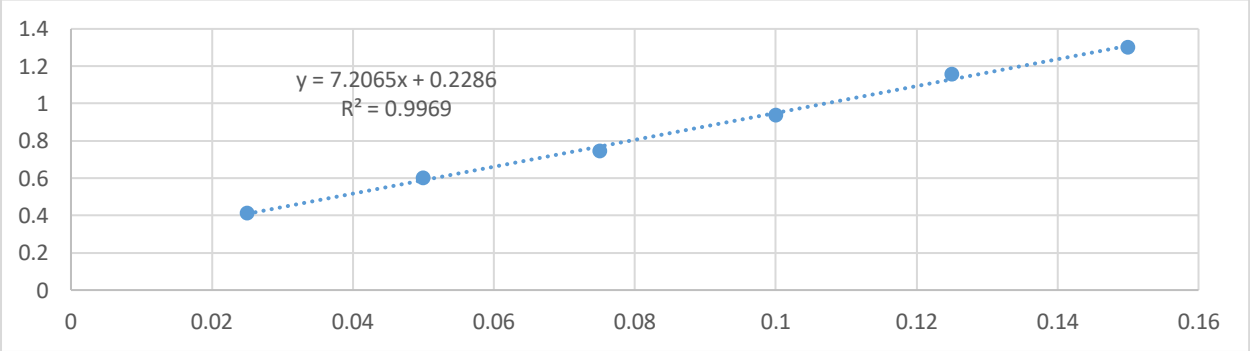
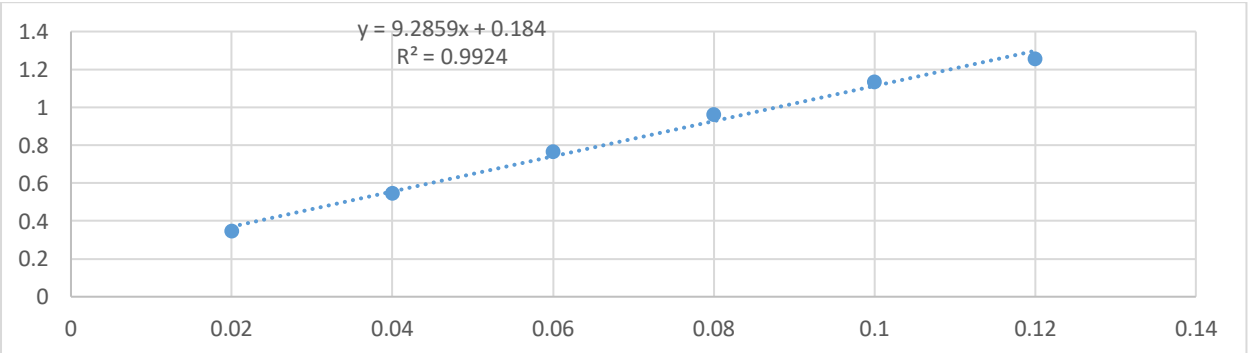


Fig 3: Standard calibration curve for total flavonoid content for standard quercetin



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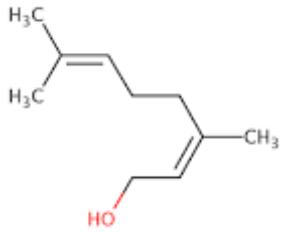
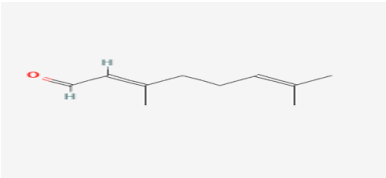
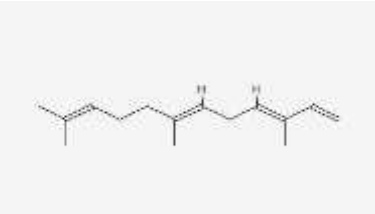
Fig 4: Standard calibration curve for total phenolic content for standard gallic acid

GC-MS Analysis of ethanolic *Mucuna pruriens* seeds extract

The GC-MS Chromatogram of the ethanolic seed extract of *M. pruriens* revealed the presence of five major compounds namely; Cyclododecane, Nerol, methyl ether, Bicyclo [10.1.0] tridec-1-ene, (R)-(-)-14-Methyl-8-hexadecyn-1-ol and 9,12-Octadecadienoic acid, ethyl ester.

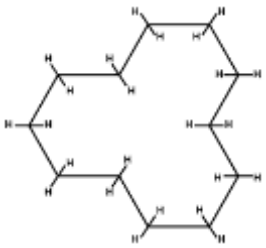
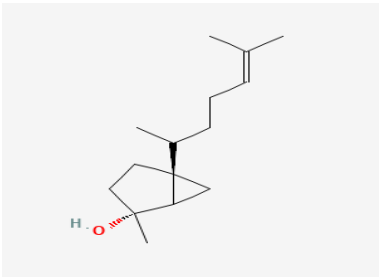
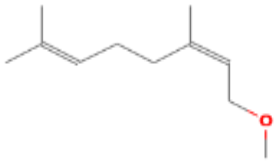
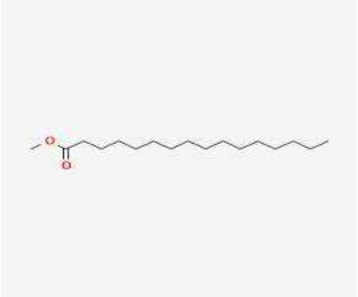
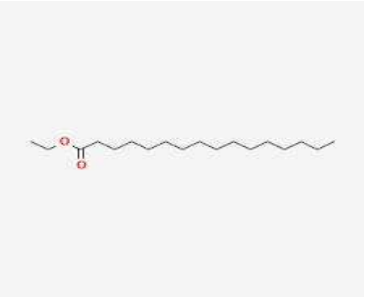
The retention time, name of the compound, molecular formula, molecular weight and chemical structure are discussed in (Table 3). Figure 5 shows the gas chromatogram of the extract and the mass spectra of the compounds are presented in Figure. 6A, 6B, 6C, 6D and 6E. Major pharmacological activities of identified phytochemicals from *M. pruriens* seed extract were given in Table 4.

Table 3: GC-MS compounds found in the ethanol extract of *M. pruriens* seed extract

RT	Area %	Name of a compound	Molecular formula	Molecular weight	Chemical structure
8.8717	1.1661	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	$C_{10}H_{18}O$	154.2493	
9.0891	0.8533	Citral	C10H16O	152.24	
12.179	1.1888	Alpha-Farnesene	C15H24	204.35	

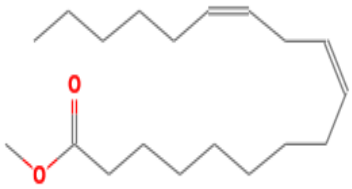
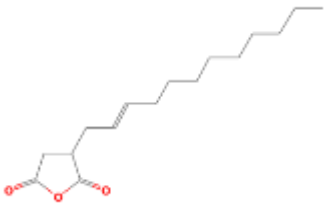
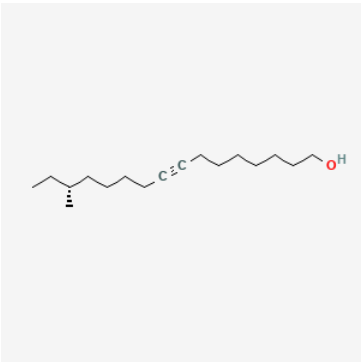
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13.249	8.832	Cyclododecane	*C12H24*	168.32	
13.2776	4.2003	7-epi-cis-sesquisabinene hydrate	C15H26O	222.37	
13.7125	7.2137	Nerol, methyl ether	$C_{11}H_{20}O$	168.2759	
15.0228	2.7207	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	
15.4977	2.5325	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	

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16.0356	1.4272	Pentacosane	$C_{25}H_{52}$	352.6804	
16.1901	7.4801	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	
16.2301	1.1766	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	266.3758	
16.6192	2.9397	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.4986	
16.7909	16.7665	Bicyclo[10.1.0]tridec-1-ene	C13H22	178.31	
16.8424	5.9788	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	C17H32O	252.4	

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Table 4: Major pharmacological activities of identified phytochemicals from *M. pruriens* seed extract

Biochemical compound	Biological properties	References
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	Antimicrobial	[22]
Citral	Antimicrobial Anticancer anti-inflammatory	[23] [24] [25]
Alpha-Farnesene	Plants natural defense mechanism Antimicrobial, antiviral Antioxidant	[26] [27] [28]
Cyclododecane	Antimicrobial, antioxidant	[29]
7-epi-cis-sesquisabinene hydrate	Anticancer	[30]
Nerol, methyl ether	-	-
Hexadecanoic acid, methyl ester	Antimicrobial	[31]
Hexadecanoic acid, ethyl ester	Antimicrobial	[32]
Pentacosane	Antimicrobial	[33]
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anticancer Antimicrobial Raises VLDL And Lowers HDL Cholesterol	[34] [35] [36]

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2-Dodecen-1-yl(-)succinic anhydride	Antioxidant Antimicrobial and antifungal	[37] [38]
9,12-Octadecadienoic acid, ethyl ester	Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic	[39]
Bicyclo[10.1.0]tridec-1-ene	no study has yet reported any biological characteristics	[40]
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	-	-

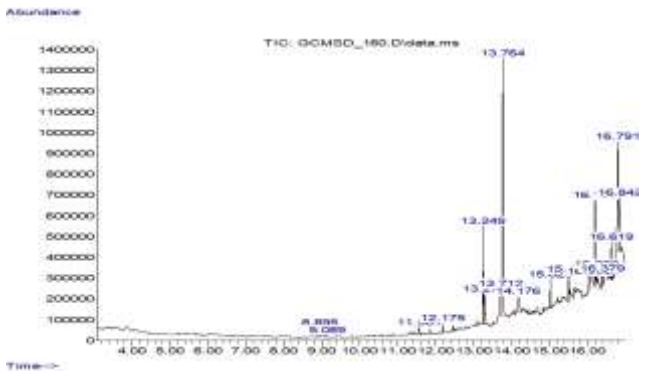


FIG. 5: Gas chromatogram of ethanolic extract of *M. pruriens* seed

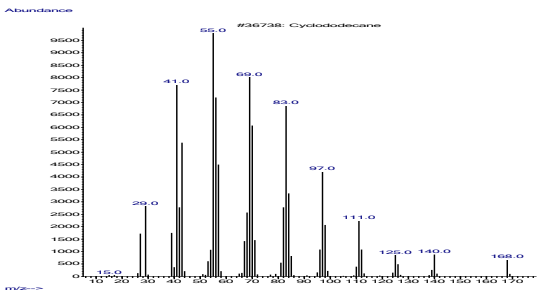


Fig. 6A: Mass spectra of Cyclododecane

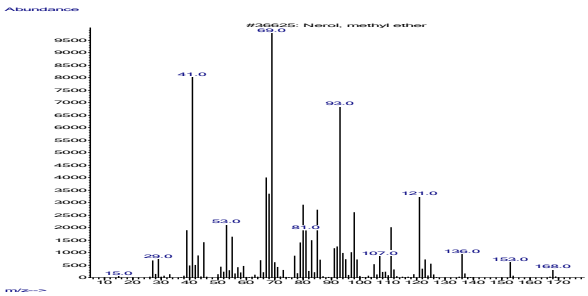


Fig. 6B: Mass spectra of Nerol, methyl ether

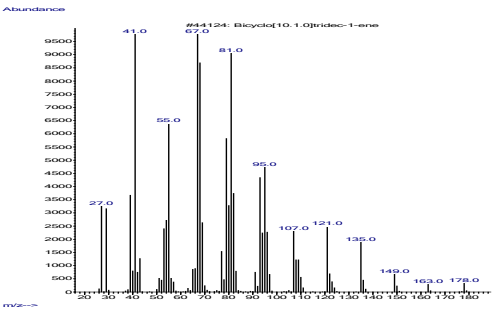


Fig. 6C: Mass spectra of Bicyclo[10.1.0]tridec-1-ene

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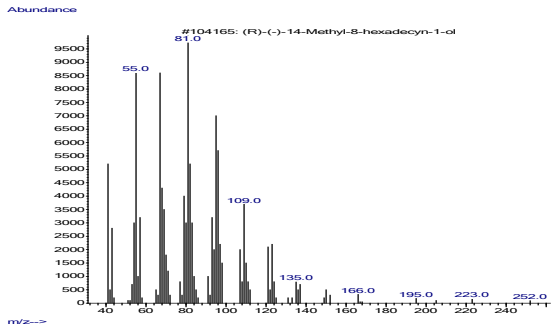


Fig. 6D: Mass spectra of (R)-(-)-14- Methyl-8-hexadecyn-1-ol

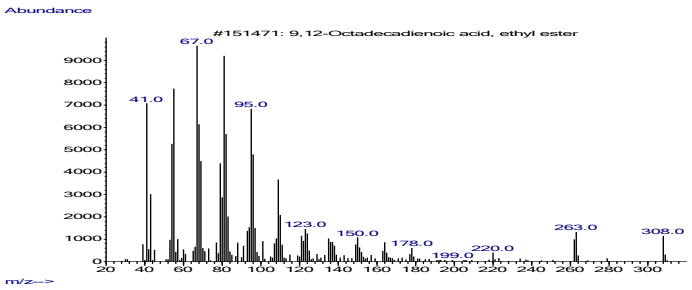


Fig. 6E: Mass spectra of 9,12-Octadecadienoic acid, ethyl ester

Discussion

The qualitative phytochemical screening of the Ethanolic seed extract of *M. pruriens* showed the presence of phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, saponins, steroids and reducing sugars as shown Table 1. The results of the present study are consistent with previous findings on *M. pruriens.* For instance, this study supports the findings of [41] reported that ethanolic seed extract of mucuna contain alkaloids, flavonoids, steroids, amino acids, triterpenoids, saponin, tannin, protein, carbohydrates and phenol. Kavitha [42] also reported that *M. pruriens* seed extract contains alkaloids, phenols, flavonoids, terpenoids, coumarin, steroids, saponins and tannins. While phytochemical evaluation of the methanolic extract of *M. pruriens* seeds by [43] noted the absence of saponins and triterpenoids. This variation in the phytochemical constitution may be attributed to the fact that genomic composition, agroecology, agronomy, postharvest technology, storage conditions and widespread use of pesticides [44].

Alkaloids are naturally occurring compounds containing nitrogen atoms and are known for their pharmacological effects and used as medications and recreational drugs [45]. Flavonoids extracted from medicinal plants are water soluble antioxidants and free radical scavengers which can prevent oxidative cell damage and have strong anticancer property [46]. Terpenes protect the plant from abiotic stress such as high light intensity, high temperature, and oxidative stress [47]. They are also useful nutrients for human consumption and are used as chemotherapeutic agents for their antitumor property [48]. The glycosides are important in lowering blood pressure [49].

The higher amount of total tannin is important for several reasons, including antioxidant activity, potential health benefits and their role in plant defense mechanisms [50]. Consumption of diets rich in polyphenols provides defense against the development of cardiovascular diseases, cancer, diabetes, osteoporosis and neurodegenerative diseases [51]. Flavonoids had been reported to exert wide range of biological activities which includes anti-inflammatory, antibacterial, antiviral and anti-allergic activity [52].

Among the identified phytocompounds, Hexadecanoic acid, methyl ester, Hexadecanoic acid, ethyl ester, Pentacosane, 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- have antimicrobial activities [22,31,32,33]. 9,12-Octadecadienoic acid, ethyl ester has the property of Hepatoprotective, antihistaminic, hypocholesterolemic and anti-eczemic. [39] 7-epi-cis-sesquisabinene hydrate was reported for anticancer property [30].

Citral is an essential component in the manufacturing of scents, citrus chemicals, cosmetics, food and pharmaceutical products [53]. It possesses antimicrobial activity, anticancer and anti-inflammatory properties [23,24,25]. Cyclododecane's industrial significance stems from its use as a crucial intermediate in producing Nylon 12 and other polymers. It has also unique sublimation properties for temporary applications like conservation, for flame retardants, detergents, and lubricating oils, temporary binder and fixative [54]. On the other hand, hexadecanoic acid, methyl ester act as a softener and plasticisers [55]. 2-Dodecen-1-yl(-)succinic anhydrides used as a curing agent for epoxy resins, providing excellent physical-chemical and electrical properties to the cured resins [56]

The compound 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester found in the extract exhibits inhibitor activity against catecholamine O methyl transferase and methyl guanidine [57]. Which was also reported by the earlier worker in the methanolic seed extract of *M. pruriens* [58]. This indicates the antioxidant activity of the said compound. MG has also been implicated in brain dysfunctions, such as epilepsy [59,60].

Hexadecanoic acid, ethyl ester has flavoring property, hypocholesterolemic activity, nematicide and anti-androgenic activity [61]. Hexadecanoic acid and ethyl ester are well known antioxidant compound was also reported to be found in *Mucuna pruriens* seed [62]. However, the rest compounds obtained in this study were not reported in previous studies of this species. This variation might be due to factors like location, climate, how mature the plants are, and the type of soil they grow in. These elements can influence the quality of the herbal ingredients in a particular species and also cause significant differences in the bioactive compounds found in the plants [14].

Conclusion

The results of the present study demonstrated that ethanolic seed extract of *M. pruriens* seeds possess secondary metabolites such as Alkaloids, flavonoids, phenol, saponin, steroids, tannin, terpenoids. From GC-MS analysis different bioactive compounds having antimicrobial, anticancer, antioxidant and anti-inflammatory properties were identified. Further studies are recommended to quantify the L-DOPA content of *M. pruriens* seeds

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[DOI: 10.66224/pbp.8.3.2]

Author Contributions

Conceptualization, Y. G. and H.A.; investigation and data curation, Y.G.; writing—original draft preparation, Y.G.; writing—review and editing, Y.G and H.A.
All authors have read and agreed to the published version of the manuscript.

Funding

No funding allocated for this research work
Institutional Review Board Statement: Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Acknowledgments

We would like to express our gratitude to all gene bank case team members for their support, and especial thanks to Dr Tesfaye Awas, involved in the fieldwork.
Conflicts of Interest: The authors declare no conflict of interest.

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