Phytochemical analysis and bioactive compound identification using GC-MS of *Mucuna melanocarpa* Hochst. ex A.Rich. ethanolic seed extract

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***Mucuna melanocarpa* is a tall climbing plant, belonging to the family Fabaceae and is endemic to Ethiopia. While the genus *Mucuna* is known for its nutritional and medicinal properties, the understudied species *M. melanocarpa* remains largely unexplored. Therefore, this study aimed to investigate the qualitative and quantitative phytochemical analyses and to identify bioactive compounds using the GC–MS technique. The phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, terpenoids saponins, tannins and proteins. The GC-MS analysis showed the presence of two major compounds, octacosane and geraniol known for their antimicrobial, antioxidant, anti-inflammatory, and anti-tumor properties.**

Keywords: *Mucuna melanocarpa;* phytochemical screening; GC-MS; octacosane; geraniol

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1. INTRODUCTION

Mucuna melanocarpa is an endemic plant to Ethiopia. The species occurs on steep, rocky slopes, in woodland, and along the borders of montane forest (Moura et al., 2024). *M. melanocarpa* is a tall climber, at least 10 m long; stems are pubescent. Leaflets rhomboid or broadly ovate, the laterals very asymmetrical, up to 10-18 x 8-14 cm, subglabrous to appressed pilose above, sparsely pubescent to densely appressed silvery pilose beneath. The inflorescences are pendulous, forming many-flowered false racemes. The calyx is densely velvety, covered with appressed orange-red bristly hairs that cause intense skin irritation and itch due to presence of a chemical called mucunain (Hedberg and Edwards, 1989).

All parts of the *Mucuna* plant are recognized for their medicinal properties (Caius, 1989; Warrier, 1993). Different species of *Mucuna* have been found to contain several beneficial phytochemicals (Morris, 1999). The plant contains a range of compounds in its pods, seeds, leaves, and roots, including bufotenine, choline, N, N-dimethyltryptamine, 5-hydroxyindole-3-alkylamines, indole-3-alkylamines, 5-methoxytryptamine, serotonin and beta-carboline (Ghosal et al., 1971). In Ayurvedic and traditional medicine, the roots of *Mucuna* are used to treat conditions such as constipation, kidney diseases, difficulty in urination, painful menstruation, absence of menstruation, elephantiasis, edema, nerve disorders, tuberculosis, ulcers, worm infestations, fever, and delirium (Szabo, 2003). The leaves are considered to have aphrodisiac and anthelmintic properties and are used for treating ulcers, inflammation, worm infestations, headaches, and overall weakness. The hairs found on *Mucuna* pods are mixed with honey and used as a remedy for expelling worms. A paste made from these hairs is also used as a stimulant and a mild irritant (Sastry and Kavathekar, 1990).

The diversities in genus *Mucuna* needs further intensive attention and research because of its potential in pharmaceutical, nutraceutical and cosmeceutical applications. It is feasible to develop extracts that maintain an acceptable balance of nutritional and bioactive components, making them suitable for use in the development of traditional medicines (Baruah et al., 2025).

In Ethiopia, the seeds of *M. melanocarpa* have traditionally been used as a medicine for nerve tonic due to its ability to uplift one's mood and enhance a sense of emotional well-being. While the genus *Mucuna* is known for its nutritional (e.g., *Mucuna pruriens*) and medicinal properties, the understudied species *M. melanocarpa* remains a largely unexplored reservoir of unique bioactive compounds. Its specific phytochemical profile, nutritional efficacy, and antimicrobial spectrum have not been systematically investigated using modern biotechnological tools. Therefore, the aim of this study was to determine the

qualitative, quantitative phytochemical analysis and bioactive compound identification using GC-MS. As far as we know this is the first study to characterize this plant species for the presence of bioactive compounds with various effects on biological systems.

2. MATERIAL AND METHODS

2.1. Plant materials

The collected seeds of *M. melanocarpa* were taken out from the pods and ground in to fine powder using, grinder and stored for further analysis. Ten g of *M. melanocarpa* seed powder was added into a conical flask containing 100 mL of ethanol and kept for 72 hours in an incubator shaker at 37 ℃ after which the solution filtered using Whatman filter paper No. 1. The filtrate was dried and stored for further analysis.

2.2. Qualitative phytochemical analysis

Qualitative phytochemical screening was performed according to standard methods given by Brain and Turner (1975) and Evans (1997).

2.2.1. Test for alkaloids

The sample extract was added to dilute HCl and then filtered. The filtrate was used for the detection of alkaloids.
Mayer' s test: *Two* drops of Mayer's reagent were added to the filtrate. Formation of a yellow precipitate is an indication of positive result for alkaloids.
Wagner's test: Two drops of Wagner's reagent were added to the filtrate. A reddish-brown precipitate is an indication of positive result for alkaloids.

2.2.2. Test for flavonoids

Lead acetate test: Two drops of lead acetate solution were added to 5mg of the ethanolic seed extract in a test tube. Formation of yellow color precipitate is an indication of positive result for flavonoids.
H_2SO_4 test: Two drops of H_2SO_4 were added to the seed extract (5 mg). Formation of orange color is an indication of positive result for flavonoids.

2.2.3. Test for steroids

Acetic anhydride (3 mL) was added to the extract (5 mg) and H_2SO_4 (2 mL) was carefully added to the solution. A color change from violet to blue or green confirm positive result for steroids.

2.2.4. Test for terpenoids

Salkowski's Test: Ethanolic seed extract (5 mg) was combined with chloroform (2 mL) in a test tube and concentrated H_2SO_4 (3 mL) was added drop by drop to form a layer. A reddish-brown color in the inner face is an indication of positive result for terpenoids.

2.2.5. Test for phenols

Ferric chloride test: Two drops of ferric chloride solution were added to the extract (7 mg) in a test tube. Bluish black color is an indication of positive result for phenols.
Lead acetate test: Two drops of lead acetate solution were added to the extract (7 mg) in a test tube. Formation of yellow color precipitate is an indication of positive result for phenols.

2.2.6. Test for saponins

Ethanolic seed extract (1 mg) was shaken with distilled water (5 mL) in a test tube. Appearance of foaming is an indication of positive result for saponins.

2.2.7. Test for tannins

A small amount of extract was added to water and heated on a water bath then ferric chloride was added in to the filtrate. A dark green color is an indication of positive result for tannins.

2.2.8. Test for protein and amino acids

Biuret test: Ethanolic seed extract (1 mg), equal volume of NaOH (40%) solution and few drops of copper sulphate (1%) solution were added in a test tube. The appearance of violet color is an indication of positive result for protein.
Ninhydrin test: Ethanolic seed extract (1 mg) was taken and few drops of Ninhydrin reagent (0.2%) were added and heated. Formation of pink or purple color is an indication of positive result for proteins, peptides or amino acids.

2.3. Quantitative screening of bioactive component

After qualitative phytochemical estimation, quantitative phytochemical estimation was performed to determine total phenolic content, as well as total flavonoids and total tannin content.

2.3.1. Determination of total phenolic content

The total phenolic content was estimated using Folin–Ciocalteau reagent method. Three levels of concentrations of extract have been prepared and then 150 µL have been taken from each concentration and combined with 1 mL of Folin–Ciocalteau reagent and 1 mL of Na_2CO_3 2% (w/v). The absorbance of the samples was taken at 765 nm. The results were defined in mg of gallic acid equivalent (GAE) per g of plant powder (Harborne, 1973). To determine the phenolic content, the absorbance of the sample was measured and compared against a gallic acid standard curve.

2.3.2. Determination of total flavonoid content

The total flavonoid content was calculated using a colorimetric assay. A sample (1mL) was combined with distilled water (2 mL) and 15% of $NaNO_2$ solution (0.15 mL) was added. After 6 minutes, 10% of an $AlCl_3$ solution (0.15 mL) was poured and left to stand for 6 minutes, then 2 mL of 4% NaOH solution was added to the solution. Instantly, water was added until the final volume reaches up to 5 mL and left to stand for another 15 minutes. The absorbance of the samples was taken at 510 nm. The result was expressed as quercetin equivalents (QE) per gram of sample (Chang et al., 2002).

2.3.3. Determination of total tannin content

Sample extract (1 mL) was added to 75 mL water, followed by folin denis reagent (4 mL), and 9 mL of sodium carbonate solution and the mixture was then diluted to 90 mL with water and shaken well. The absorbance was read at 725 nm. A blank was prepared using water. A standard graph was prepared by using 100 mg tannic acid (Nair et al., 2015). The absorbance of the sample was compared against the standard curve to find its concentration in µg/mL, which were then back-calculated to the original weight of the dry plant extract to give the final unit (mg TAE/g).

2.4. GC-MS Analysis

Ethanolic extract was also analyzed 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). Instrument control and data processing were performed using Xcalibur™ software (Thermo Fisher Scientific, Waltham, MA, USA). Identification of phytoconstituents was verified based on relative retention time and peak area, using the NIST and Wiley

mass spectral libraries integrated into the GC–MS system, Agilent technology 7820A GC and 5977E MSD systems.

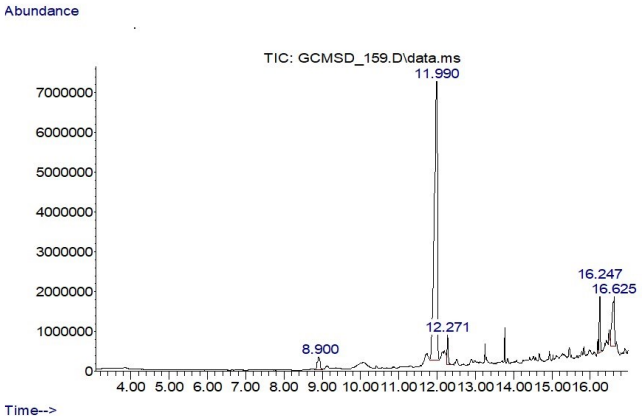


Figure 1. The gas chromatogram of the ethanolic seed extract of *M. melanocarpa*

2.4.1. 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.

3. RESULTS

3.1. Qualitative phytochemical analysis

The result of the phytochemical analysis indicated that ethanolic seed extract of *M. melanocarpa* revealed the presence of flavonoids, tannins, alkaloids, terpenoids, saponins, steroids and proteins as shown in Table 1.

3.2. Quantitative phytochemical analysis

Ethanolic seed extract of *M. melanocarpa* was shown to be a rich source of secondary metabolites, containing phenolic compounds (1.555 ± 0.001 mg GAE/g), flavonoids (1.739 ± 0.007 mg QE/g), and tannins (6.176 ± 0.757 mg TAE/g), as summarized in Table 2. Among these phytochemicals, tannins were the most abundant, followed by flavonoids and phenols.

3.3. GC-MS Analysis of ethanolic Mucuna melanocarpa seed extract

The GC-MS chromatogram of the ethanolic seed extract of M. melanocarpa showed five peaks, with two major compounds that might be solvent specific. Among the identified compounds, octacosane exhibited the highest peak area (10.45%), followed by geraniol (3.02%).

The retention time, compound name, molecular formula, molecular weight, chemical structure and the percent peak area, are presented in Table 3. Figure 1 shows the gas chromatogram of the extract with five peaks, including two identified compounds, while the mass spectra of these compounds are presented in Figure 2A and 2B.

4. DISCUSSION

The ethanolic extract of *M. melanocarpa* seeds contained flavonoids, steroids, alkaloids, tannins, carbohydrates, and amino acids. This report is in good agreement with previous studies conducted on the *Mucuna* genus. For instance, Krishnaveni and Hariharan (2017) reported that aqueous extract of *Mucuna pruriens* contains flavonoids, steroids, alkaloids, tannins, carbohydrates, and amino acids. However, the study conducted by Tavares et al. (2015) using hydroalcoholic extract showed that there were no alkaloids and tannins, but a weak presence of steroids and saponins, and a moderate presence of flavonoids. The phytochemicals in the seeds may be linked to their therapeutic properties. According to Varadarajan et al. (2015), the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins are glycosides of both triterpenes and steroids and have hypotensive and cardiodepressant properties.

As noted by D'Mello (1995), anti-nutritional compounds present in plants contribute to resistance against insects and diseases. Polyphenols are key phytochemicals having free radical scavenging activities (Bravo et al., 1998). According to Beta et al. (2005) flavonoids have anti-oxidant, anti-mutagenic and anti-cancerogenic activity. Mir et al. (2013) reported that steroids and saponins have major functions in central nervous system activities.

Table 1. Qualitative phytochemical constituents of *Mucuna melanocarpa* ethanolic seed extract

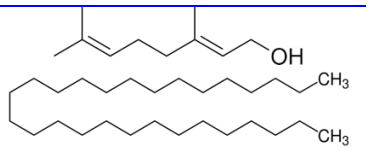
S. No	Phytochemical constituent	*Mucuna melanocarpa* seeds extract
1	Alkaloids	Present
2	Flavonoids	Present
3	Tannins	Present
4	Terpenoids	Present
5	Saponins	Present
6	Steroids	Present
7	Protein and amino acid	Present

Table 2. Quantitative phytochemical constituents of *Mucuna melanocarpa* ethanolic seed extract

Phytochemicals	Content
Total phenolic	1.555 ± 0.001 mg GAE/g
Total flavonoid	1.739 ± 0.007 mg QE/g
Total tannin	6.176 ± 0.757 mg TAE/g

The result of this study indicated that tannins are the dominant active components found in this plant. Tannins are known for the antiviral, antibacterial, and antitumor properties. In addition, tannins have been reported to selectively suppress the replication of HIV virus (Ezeonu and Ejikeme, 2016). The study conducted by Theansungnoen et al. (2022) revealed that ethanolic seed extract of *Mucuna* species contains flavonoids and phenols.

Table 3. Compounds identified in the ethanolic seed extract of *M. melanocarpa* by GC-MS analysis

PK	RT	Area Pct	Library/ID	Formula	Molecular weight	Chemical structure
1	8.9003	3.0233	Geraniol	$C_{10}H_{18}O$	154.25	
5	16.625	10.4568	Octacosane	$C_{28}H_{58}$	394.7601	

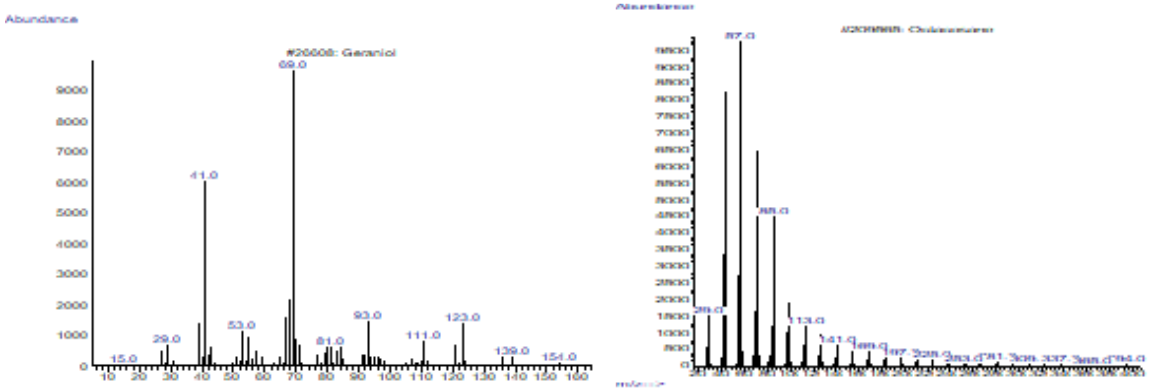


Figure 2. A. Mass spectra of geraniol. **B.** Mass spectra of octacosane

Table 4. Pharmacological activities of ethanolic seed extract of *Mucuna melanocarpa*

Biochemical compound	Biological properties	References
Geraniol	Antimicrobial, antioxidant, anti-inflammatory	(Fajdek et al., 2024)
Octacosane	Antioxidant, anti-inflammatory larvicidal effects Anti-tumor	(Tanod et al., 2019) (Rajkumar and Jebanesan, 2004) (Figueiredo et al., 2014)

Octacosane is a straight-chain alkane containing 28 carbon atoms. It has a role as a plant metabolite and known for its potential health benefits (National Center for Biotechnology Information, 2023). Studies have suggested that octacosane may promote wound healing due to their potent free radical scavenging, hydroxyproline and glutathione action, Table 4. (Balachandran et al., 2023) .

Geraniol, another prominent constituent of *M. melanocarpa* seed, is a promising chemoprevention agent for cancer. Other biological activities such as antimicrobial, anti-oxidant, and anti-inflammatory properties have been reported by Fajdek et al. (2024).

This study represents the first attempt to isolate and characterize bioactive compounds from the seeds of this plant species.

5. CONCLUSION

The results of this study indicate that ethanolic seed extract of *M. melanocarpa* contains alkaloids, flavonoids, steroids, terpenoids saponins, tannins and proteins, which possess antioxidant, anti-microbial and anti-inflammatory activities. GC-MS analysis revealed the presence of octacosane, and geraniol which are known for their antimicrobial, anticancer, antioxidant and anti-inflammatory properties. Further research on this plant is necessary, as the available data are scarce, primarily because *M. melanocarpa* has not yet been extensively investigated.

AUTHOR CONTRIBUTIONS

Conceptualization, Y. G. and H.A.; investigation and data curation, Y.G., H.A and A.S; writing—original draft preparation, Y.G.; writing—review and editing, Y.G, H.A and A.S.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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