Phytochemical and Antimicrobial Studies on the Seeds of
*Mucuna Monosperma* DC

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Abstract

The phytochemical study of the seeds of *Mucuna monosperma* DC, revealed the presence of flavonoids, sterols, triterpenoids. The separated compounds were identified based on their melting points, IR, ¹H NMR and mass spectral data, such compounds were acacetin, luteolin, b-sitosterol, stigmasterol, ursolic acid and betulinic acid. The antimicrobial activity of chloroform and methanolic extract of seed powder was investigated. The methanolic extract exhibited marked antibacterial activity against gram positive, gram negative organisms and fungi.

Key words

*Mucuna monosperma*, flavonoids, triterpenoids, antimicrobial activity

Introduction

The plant, *Mucuna monosperma* DC. (Fabaceae) is a large woody perennial large climber. This plant commonly known as Pedda dulagondi, enugudulagondi in Telugu, Khatarangi in Sanskrit, and Negro been in English (1, 2). It is distributed in tropical India, especially in eastern Himalayas and Konkan (3). Traditionally the seeds are being used to treat the various ailments. The literature survey revealed that the seeds of *Mucuna monosperma* posses various pharmacological properties like astringent, cardiotonic, restorative, expectorant, and used in the treatment of asthma, cough and tongue infection (4). The related species such as *M. prurienns* and *M. bracteta* were reported to have insecticidal activity (5).

Earlier Mohan et al. reported the presence of amino acids like isoleucine, tyrosine and phenyl alanine (6). The seed lipid contains higher concentrations of palmitic acid and linoelic acid, behenic acid. Seeds were rich in minerals such as Na, K, Mg, Zn and Fe, anti nutritional substances, total free phenols, tannins, L-Dopa and had agglutinating activity (7-9). Hence it was felt more phytochemical and biological investigations are required on the seeds of *Mucuna monosperma*, the present study was taken up.

Materials and Methods

The plant (*Mucuna monosperma*) growing in Andhra University campus, Andhra pradesh, India was authenticated by Prof. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Vishakapatnam. A voucher specimen (Voucher number: PMB-170, deposited in: Herbarium, director: Prof. M. Venkaiah.) was deposited in the Department of Botany, Andhra University.

They were dried and powdered and defatted with n-hexane (4 x 3.5 L). The defatted material was extracted with chloroform (5 x 3 L) and methanol (4 x 3 L) successively. The chloroform,
methanol extracts were concentrated under reduced pressure and yielded extracts in the weight of 10 g and 15 g respectively. The extracts were subjected to preliminary phytochemical tests. Chloroform extract gave positive identification tests for the Liebermann-Burchard (L.B) and shinoda’s test (SHT). It also showed two spots on TLC. Hence it was subjected to column chromatography which yielded two compounds designated as MMS-01 and MMS-02. The methanolic extract was dark brown colour and showed positive colour reactions with ferric chloride test, SHT and also L.B. test. In paper chromatographic examination two spots were identified under UV. Hence it was subjected to column chromatography which yielded four compounds designated as MMS-03, MMS-04, MMS-05 and MMS-06. Melting points of the compounds were determined by boietus micro melting apparatus. The IR (KBr) and UV spectra were recorded on Shimadzu IR and UV spectrophotometers. The 1H NMR spectra was recorded on JEOL Ex 90 FT NMR spectrometer by using CDCl3 and DMSO-d6 with TMS as internal standard. The identified compounds were confirmed by comparison with authentic samples.

Characterization of the Compounds

**MMS-01:** It was crystallized as fine colourless feathery needles from 25% hexane-chloroform fraction, melting point 134-136°C. It showed positive color reaction, play of colours (pink-blue-green) with L.B test for sterols. Its IR spectral data and other properties indicated that it was a b-sitosterol (Fig-1).

**MMS-02:** It was crystallized as colourless needles from 50% hexane-chloroform fraction, melting point 169-170°C. It showed play of colours with L.B test for sterols. IR spectrum exhibit the bands at 1172, 1132, 1072, 991, 971 and 935cm⁻¹. Based on the above data it was identified as stigmasterol (Fig-1).

**MMS-03:** It was crystallized as light yellow needles from chloroform-methanol fraction, melting point 258-260°C. It gave dark green colour with ferric chloride test and orange red colour in SHT, suggested that it was a 5-hydroxy flavone. In UV spectrum a free 7-hydroxyl group was indicated by 9 nm bathochromic shift of the low wave length bond on the addition of NaOAc. The 1H NMR (CDCl3) spectral data in d3.85 (3H-S-4'-OCH3), 6.40 (1H-S-C-3H), 6.50 (1H-d-[J=2.2]-C-^H), 6.60 (1H-d-[J=23.2]-C-8H), 7.26 (2H-d-[J=8.5]-C-3'H, 5'H) and 9.83 (2H-d-[J=8.5]-C-2' H, 6' H). From the above data it was identified as 5, 7-dihydroxy-4'-methoxy flavone, acacetin (Fig-1).

**Fig. 1:** Chemical structures of compounds isolated from the seeds of *Mucuna monosperma*.
MMS-04: It was obtained as yellow needles from chloroform-methanol fraction, melting point 333-334°C. It gave green colour with ferric chloride, pink colour with SHT indicating that it was a 5-hydroxy flavone. In UV spectrum a large bathochromic shift of 52 nm in band I with NaOMe indicated a free 4'-hydroxy group and band II shift of 16 nm with NaOMe suggested the presence of 7-hydroxyl group. At 36 nm in band I with AlCl3 / HCl confirmed the 5-hydroxyl group, at 21 nm with NaOAc / H3BO3 and 41 nm with AlCl3 in band I infrared 3',4'-dihydroxy groups suggested that it was a 5, 7, 3', 4'-tetrahydroxy flavone. It was compared with an authentic sample as a luteolin (Fig-2).

MMS-05: Fine powder with melting point 276-278°C was obtained from chloroform-methanol fraction. It showed positive L.B. test. IR spectrum showed the absorption bands at 3490, 1703, 1660, 1386, 1380, 1018 cm⁻¹ and its ¹H NMR (CDCl₃) spectrum of the acetate exhibits the peaks at δ 8-1.00 (21H-M-7 x CH₃), 1.98 (3H-s-OCOCH₃), 4.40 (1H-d-olefinic proton), 5.20 (1H-m-e-3H) and 11.20 (1H-s-COOH). From the above data it was identified as ursolic acid (Fig-2).

MMS-06: Whitish fine needles with melting point 278-280°C were obtained from methanolic extract. It gave pink colour with L.B. test. IR spectrum displayed the peaks at 3462, 1692, 1640, 1388 and 1380 cm⁻¹. The ¹H NMR (DMSO-d₆) spectrum showed the peaks at δ 0.83-1.60 (15H-S-5 x CH₃), 1.70 (3H-S-C=CH₃), 1.96 (3H-S-OCOCH₃), 3.60 (3H-S-OCOCH₃) and 4.65 (2H-d [J=8]-vinyl protons). Based on the above data it was identified as betulinic acid (Fig-2).

**Antibacterial and Antifungal Activity**

**Antibacterial activity:** The bacterial strains obtained from department of Biotechnology, Andhra University, Vishakapatnam. The chloroform and methanolic extracts were obtained successively from defatted seeds of *Mucuna monosperma* and tested to screen the antibacterial activity against *Bacillus pumilis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris*. The extracts were dissolved in dimethyl sulphoxide to determine the activity. Cup plate agar diffusion method (10) was used to determine the zone of inhibition of the extracts. For comparison the Ampicillin (1µg/ml) was used as a standard.

Two drops (0.05 ml) of solution of the extracts of each concentration (100mg/ml, 300mg/ml) were placed in the cups by means of sterile
pipettes. The plates thus prepared were left for one hour at room temperature for diffusion. After incubation for 24 hours at 31°C the plates were examined for the zones of inhibition.

**Antifungal activity:** The fungal strains obtained from department of Biotechnology, Andhra University, Vishakapatnam. The chloroform and methanolic extracts tested for antibacterial activity were also studied for antifungal activity against Aspergillus niger, Rhizopus oryzae. The antifungal activity was tested in the same way as antibacterial activity by using the fungal organisms and for comparison the Nystatin (100 mg/ml) was used as a standard.

**Results and Discussion**

Chemical analysis of *Mucuna monosperma* seeds revealed the presence of sterols, triterpenes and flavonoids. The flavonoids like acacetin and luteolin possess tri (or) tetra substitution with 5-OH group in their molecular structure. This type of flavonoids is usually seen in 50% legume compounds. Except sterols the other isolated compounds were being recorded for the first time from *Mucuna* seeds. The methanolic extract showed marked activity against Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus subtilis and the results were shown in Table-1. In the case of antifungal activity methanolic extract showed moderate activity against Aspergillus niger, Rhizopus oryzae and the results were shown in Table-2. The chloroform extract did not show any antibacterial and antifungal activity. The activity of methanolic extract may be due to the presence of MMS-03 to MMS-06.

In conclusion our results have shown that seed powder of *Mucuna monosperma* possess antibacterial and antifungal activity. The seeds seem to have a promising value for the development of potent phytomedicine for microbes. Further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antimicrobial effect of *Mucuna monosperma*.

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<th>Table1: Antibacterial activity of Extracts of the <em>Mucuna monosperma</em> seeds</th>
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<tr>
<td>Test organisms and Zone of inhibition (mm)</td>
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<td>S.No. extracts &amp; standard</td>
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Cup diameter = 6mm

**B.P = Bacillus pumilis, B.S = Bacillus substilis, S.A = Staphylococcus aurens,**

**P.A = Pseudomonas aeruginosa, E.C = Escherichia coli and P.V = Proteus vulgaris.**

### Table 2: Antifungal activity of Extracts of the *Mucuna monosperma* seeds

<table>
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<th>S.No.</th>
<th>Extracts and standard</th>
<th>Test organisms and Zone of inhibition (mm)</th>
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<td>Aspergillus niger</td>
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<td>2.</td>
<td>CHCl3 extract (300 mg/ml)</td>
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<td>3.</td>
<td>Methanolic extract (100 mg/ml)</td>
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<td>4.</td>
<td>Methanolic extract (300 mg/ml)</td>
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<td>5.</td>
<td>Nystatin (100 µg/ml)</td>
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Cup diameter = 6mm

### References


