ABSTRACT OF THE THESIS

The results of phytochemical investigations and biological evaluation on three medicinal plants namely \textit{Mussaenda roxburghii} (Hook. F.), \textit{Dillenia pentagyna} (Roxb.) and \textit{Sarcochlamys pulcherrima} (Roxb.) are presented in the Ph. D. thesis.

The Thesis is consisting of four parts:
PART-1: CHEMICAL CONSTITUENTS OF \textit{Mussaenda roxburghii}
PART-2: CHEMICAL CONSTITUENTS OF \textit{Dillenia pentagyna}
PART-3: CHEMICAL CONSTITUENTS OF \textit{Sarcochlamys pulcherrima}
PART-4: MATERIALS AND METHODS

Publications

Parts 1-4 have been subdivided in the sections as follows:

PART-1: Section-1.1: Chemical Constituents of \textit{Mussaenda roxburghii}
Section-1.1.1: A brief review of phytochemicals of \textit{Mussaenda} species.
Section-1.1.2: Isolation and structure elucidation of MR-1
Section-1.1.3: Isolation and structure elucidation of MR-2
Section-1.1.4: Isolation and structure elucidation of MR-3
Section-1.1.5: Isolation and structure elucidation of MR-4
Section-1.1.6: Isolation and structure elucidation of MR-5
Section-1.1.7: Isolation and structure elucidation of MR-6

Section-1.2: Biological studies of \textit{Mussaenda roxburghii}
Section-1.2.1: A brief review of biological studies on \textit{Mussaenda} species.
Section-1.2.2: Antibacterial activities of \textit{M. roxburghii}
Section-1.2.3: Antidiabetic activities of \textit{M. roxburghii}
Section-1.2.4: Antioxidant activities of \textit{M. roxburghii}
Section-1.2.5: Biological Evaluation of MR – 4

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PART-2: Section-2.1: Chemical Constituents of \textit{Dillenia pentagyna}
Section-2.1.1: A brief review of phytochemicals of different \textit{Dillenia} species.
Section-2.1.2: Isolation and structure elucidation of DP-1
Section-2.1.3: Isolation and structure elucidation of DP-2
Section-2.1.4: Isolation and structure elucidation of DP-3
Section-2.1.5: Isolation and structure elucidation of DP-4

Section-2.2: Biological studies of Dillenia pentagyna
Section-2.2.1: A brief review of biological studies of Dillenia species.
Section-2.2.2: Antibacterial activities of Dillenia pentagyna
Section-2.2.3: Antidiabetic activities of Dillenia pentagyna
Section-2.2.4: Antioxidant activities of Dillenia pentagyna
Section-2.2.5: Urease activities of Dillenia pentagyna

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PART-3: Section-3.1: Chemical Constituents of Sarcochlamys pulcherrima
Section-3.1.1: A brief review of phytochemicals of Sarcochlamys species.
Section-3.1.2: Isolation and structure elucidation of SPG-1
Section-3.1.3: Isolation and structure elucidation of SPG-2
Section-3.1.4: Isolation and structure elucidation of SPG-4
Section-3.1.5: Isolation and structure elucidation of SPG-5

Section-3.2: Biological studies of Sarcochlamys pulcherrima
Section-3.2.1: A brief review of biological studies of Sarcochlamys species.
Section-3.2.2: Antibacterial activities of Sarcochlamys pulcherrima
Section-3.2.3: Antidiabetic activities of Sarcochlamys pulcherrima
Section-3.2.4: Antioxidant activities of Sarcochlamys pulcherrima

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PART-4: Materials and Method
Details of isolation of all the phytochemicals from Mussaenda roxburghii, Dillenia pentagyna and Sarcochlamys pulcherrima have been discussed. Salient features of characterization of known compounds other than those discussed in the parts 1-3, have also been discussed in this part. This part has been subdivided into the following five sections:
Section-4.1: Collection of plants and preparation of extract
Section-4.2: Isolation of chemical constituents from M. roxburghii
Section-4.3: Isolation of chemical constituents from D. pentagyna
Section-4.4: Isolation of chemical constituents from S. pulcherrima
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Section-4.5. Biological evaluation of crude materials and pure compounds

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PART-1: CHEMICAL CONSTITUENTS OF *Mussaenda roxburghii*

*Mussaenda roxburghii* is distributed in the Eastern and Central Nepal at an altitude of height about 200 cm, in moist shady places of Bhutan, Bangladesh, and Myanmar and also in the North-Eastern part of India. It has anti-inflammatory, antiviral, antioxidant and antibacterial properties. Literature survey indicates that chemical and biological studies have been done only on very few species of *Mussaenda* and no phytochemical investigation has been done on the species *roxburghii*. This work focuses on the isolation and characterization of chemical constituents along with some biological activity study of *roxburghii* species. The present investigator investigated for the chemical constituents and isolated nine compounds (designated as MR-1 to MR-9) from the aerial parts of this plant. Isolation, structure elucidation of chemical constituents of *M. roxburghii* and results of some biological studies on this plant are reported in Part-1 of the thesis.

Section-1.1.2: Isolation and structure elucidation of MR-1

MR-1, MP: 158\(^0\)C, \([\alpha]_D^{25}\) -13.5 (c 0.32, MeOH) was isolated as colorless needles that analyzed for the molecular formula C\(_{11}\)H\(_{18}\)O\(_7\) by TOF-ESIMS. This formula was consistent with the \(^1\)H- and \(^13\)C-NMR data. Based on the chemical and spectroscopic evidences the structure of compound was elucidated as (4R*, 5S*, 6R*, 8S*, 9R*)-shanzhol (I). It is a new natural product. Shanzhol showed mild antibacterial activity against *S. aureus* and *E. coli* with MIC value of 100µg/mL (both) in broth dilution method.

![MR-1](image-url)
Section- 1.1.3: Isolation and structure elucidation of compound MR-2

Compound MR-2 was isolated as amorphous powder, mp 273°C from the MeOH extract of aerial parts of the *M. roxburghii* by successive column chromatography. The molecular formula of the compound was determined as C_{35}H_{60}O_{6} from its quasi-molecular ion peak at m/z 599.23 [M+Na]^{+} in TOF-ESIMS and DEPT^{13}C-NMR spectral data. The structure of the compound MR-2 was assigned as β-sitosterol-3-O-β-D-glucopyranoside. It is a known compound but it is reported for the first time from the plant *M. roxburghii*.

Section-1.1.4: Isolation and structure elucidation of MR-3

Compound MR-3 was isolated as amorphous powder, mp 77°C, *R_{f}* 0.30 in P.E-CHCl_{3} (4:1) from the MeOH extract of aerial parts of *M. roxburghii* by successive column chromatography. The molecular formula of the compound was determined as C_{46}H_{80}O_{2} from its quasi-molecular ion peak at m/z 687 [M+Na]^{+} in TOF-ESIMS and DEPT^{13}C- NMR spectral data. Compound MR-3 was established as lupeol palmitate.
Section-1.1.5: Isolation and structure elucidation of MR-4

Compound MR-4 was obtained as a white crystalline solid, mp: 258°C, $R_f$: 0.42 (CHCl$_3$-EtOAc, 3:7), $[\alpha]_D^{25}$: +30.65 (c 0.62g/100cm$^3$, MeOH). Its TOF-ESIMS showed [M+Na]$^+$ peak at $m/z$ 527.19, corresponding to the molecular formula C$_{30}$H$_{48}$O$_6$ (calcd. 504.3430). From spectral analysis the structure was identified as 2$\alpha$, 3$\beta$, 19$\alpha$, 23-tetrahydroxyurs-12-en-28-oicacid (19$\alpha$-hydroxyasiatic acid). It is a known compound but it is reported for the first time from the plant *M. roxburghii*.

![MR-4](image)

Section-1.1.6: Isolation and structure elucidation of MR-5

Compound MR-5 was isolated as colourless crystals from the n-butanol fraction of *M. roxburghii* via repeated column chromatography. MP: 223°C, soluble in water, $R_f$: 0.59 (in acetone), TOF-ESIMS of this molecule showed $m/z$ 203.66 ([M+Na]$^+$, 100). Analyzing all spectroscopic data revealed that this compound is Myo-Inositol.

![MR-5](image)
Section-1.1.7: Isolation and structure elucidation of MR-6

Compound MR-6 was isolated as amorphous powder from n-hexane fraction of the MeOH extract of *M. roxburghii* by successive column chromatography. MP: 166\(^\circ\)C, soluble in CHCl\(_3\), \(R_f: 0.41\) (in Chloroform). The molecular formula of the compound was assigned as C\(_{30}\)H\(_{48}\)O from its molecular ion peak at \(m/z\) 423.9 [M]+ in FAB MS and DEPT \(^{13}\)C-NMR spectral data. Analysis of all the spectral data suggested that MR-6 was lupenone.

![MR-6](image)

Section-1.2: Biological studies of *Mussaenda roxburghii*

In the present study my aim was to evaluate the antimicrobial, antioxidant and anti-diabetic activity of n-butanol and chloroform sub-fractions of the crude extract of *Mussaenda roxburghii* leaves and antibiofilm, anti-PCSK3 (furin) inhibition efficacy of the MR-4. The details of the study have been discussed in subsequent sections.

**Section 1.2.2: Antibacterial activities of *M. roxburghii***

Results indicated that *mussenda roxberghii* extract possess potential antibacterial activity against *S. dysenteriae* 1, *V. cholerae* non.0139 (L4), *V. cholerae* non.0139 (CSK6669) and *E. coli*. n-butanol and chloroform subfractions of the leaf extracts showed significant antibacterial activity against almost all the pathogenic microbial strains within the range of 5mm to 12mm zone inhibition with different concentration of the extract. Details of these are reported in the thesis.
Section-1.2.3: Antidiabetic activities of *M. roxburghii*

The antidiabetic activity of n-butanol and chloroform subfractions of the leaf extracts was done by $\alpha$–glucosidase inhibitory assay. The results indicated that the leaf extract have strong anti $\alpha$–glucosidase activity. Details of this study are reported in the thesis.

Section-1.2.4: Antioxidant activities of *M. roxburghii*

Antioxidant activity was done by DPPH and superoxide radical scavenging assay. The overall findings of this study indicated that *M. roxburghii* has significant antioxidant activity which can be attributed by the presence of different antioxidant components in this plant. Details of this study are reported in the thesis.

Section-1.2.5: Biological evaluation of pure compound (MR – 4):

This compound was studied for some biological activities viz. antioxidant, PCSK3 (furin) inhibition, antibiofilm and cytotoxicity. Above mentioned biological studies showed that MR- 4 has significant antibiofilm activity at sub-MIC doses and also attenuated bacterial virulent property. MR–4 also showed mild PCSK3 inhibitory effect in *in-vitro* experiment. Details of this study are reported in the thesis.

PART-2: CHEMICAL CONSTITUENTS OF *Dillenia pentagyna*

*Dillenia pentagyna* is a plant of high medicinal value employed in our indigenous system of medicines. The tribal communities of Tripura state have been using the various parts of it for the treatment of their different ailments and diseases viz. bone fracture, piles (leaf), body pain (root), diabetes, diarrhea and dysentery (bark). It is also used as a traditional medicine in the treatment of cancer suspected diseases and other stomach ailments. Due to remarkable medicinal property and inadequate works on this plant appears in the literature, further investigation on its chemical constituents was done. The present investigator investigated on the fruits and leaves of this plant and isolated four compounds and designated as DP-1 – DP-4. Isolation, structure elucidation of
these compounds and results of some biological studies on this plant are presented in Part-2 of the thesis.

Section 2.1.2.: Isolation and structure elucidation of DP-1.

Compound DP-1 was isolated as colourless needles, mp 268°C from the EtOAc subfraction of 10% H₂O-MeOH extract of *Dillenia pentagyna* leaves. Its molecular formula was assigned as C₃₂H₅₀O₄ from its molecular ion peak at \( m/z \) 499.1 \([\text{M+H}]^+\) in FAB MS and from \(^{13}\text{C}-\text{NMR}\) data. The compound DP-1 has been identified as 3-O-acetyl ursolic acid. It is a known compound, but is reported for the first time from this plant.

![Diagram of DP-1](image)

Section 2.1.3: Isolation and structure elucidation of DP-2

Compound DP-2 was isolated as white powder, mp 239°C from the EtOAc subfraction of 10% H₂O-MeOH extract of *Dillenia pentagyna* leaves. Molecular formula C₃₂H₅₂O₂; IR: \( \lambda_{\text{max}} \) (KBr): 1738, 1642 (C=C) cm⁻¹; ESI MS showed \( m/z \) 468.93 ([M+H]+, 218 (100), 203 (32), 189 (31). The \(^{13}\text{C}-\text{NMR}\) and DEPT spectra indicated the presence of carbonyl carbon (δ 170.8), one double bond (δ 120.9 and 140.1), eight methyl, an acetyl, five methines, ten methylenes and seven quaternary carbon signals. Based on the spectroscopic data compound DP-2 is identified as β-amyrin acetate.

![Diagram of DP-2](image)
Section 2.1.4: Isolation and structure elucidation of DP-3

Compound DP-3 (C$_{30}$H$_{48}$O$_{4}$) was isolated as crystalline solid, $R_f = 0.63$ in CHCl$_3$-EtOAc (3:7), mp 256$^0$C from ethylacetate sub-fraction of 10% H$_2$O-MeOH extract of Dillenia pentagyna fruits. The molecular formula of the compound was determined as C$_{30}$H$_{48}$O$_{4}$ from its quasi-molecular ion peak at $m/z$ 495.53 [M+Na]$^+$ in FAB MS and DEPT $^{13}$C- NMR spectral data. The structure of this compound was established as maslinic acid and it is a known compound but is reported for the first time from the plant D. pentagyna.

![Diagram of DP-3]

Section 2.1.5: Isolation and structure elucidation of DP-4

The compound isolated as white needles; mp 210$^0$C. The spectroscopic data were in good agreement with the reported data of lupeol. The structure was thus established as lupeol.

![Diagram of DP-4]

Section 2.2: Biological studies of Dillenia pentagyna

Traditionally the different parts of this plant were used as cancer, wound healing, diabetes, diarrhea, bone fracture, in cut and burns, abdominal pains etc. The fruits of Dillenia pentagyna as well as Dillenia indica are eaten as raw but
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not very much well known by people. These facts prompted us for evaluation of some bioactivities with different extracts of *D. pentagyna* as well as with some purified compounds. These have been discussed in the thesis under several sections.

**Section-2.2.2: Antibacterial activities of *Dillenia pentagyna***

Results obtained in the study revealed that the tested *D. pentagyna Roxb.* fruit extracts posses potential antibacterial activity against, *S. dysenteriae* 1, *V. cholerae* non.0139(L4), *V. cholerae* non.0139(CSK6669) and *E. coli*. Details of this study have been reported in the thesis.

**Section-2.2.3: Antidiabetic activities of *Dillenia pentagyna***

This study was done by $\alpha$-glucosidase assay. Experimental results showed that the leaf extract had maximum inhibition compared to that of fruit extracts. In similar set of experiments acarbose was used as positive control. Details of this study have been reported in the thesis.

**Section-2.2.4: Antioxidant activities of *Dillenia pentagyna***

DPPH radical scavenging assay:

Chloroform and *n*-butanol subfractions of fruit extract and also Leaf extract were tested for its free radical scavenging activity in DPPH radical scavenging and superoxide radical scavenging assay procedure. Details of this study have been reported in the thesis.

**Section-2.2.5: Urease activities of *Dillenia pentagyna***

Inhibition of urease activity was studied using kinetic method by using urease/glutamate dehydrogenase (GLDH) system. It is clearly demonstrated that the urease inhibition may even address *H. pylory* colonization in stomach. This was a significant observation in terms of gastrointestinal infections with respect to the tropical region. Details of this study have been reported in the thesis.

**PART-3.1: CHEMICAL CONSTITUENTS OF *Sarcochlamys pulcherrima***

*Sarcochlamys pulcherrima* (Roxb.) is also a medicinal plant and highly consumed by some ethnic tribes and castes of Assam, Tripura and other north eastern states of India. The leaves are used in the treatment of boils and fever.
blisters, eye complications, diarrhea and dysentery. Young shoots, leaves and fruits are eaten as vegetable. It is believed that eating with pork facilitate the digestion of fats. It is also claimed that *S. pulcherrima* leaves damages tape worm egg present in pork when boiled with it. In spite of its numerous medicinal properties there were no report on *S. pulcherrima* related to its chemical constituents and bioactivity. The present investigator investigated on the fruits of this plant and isolated five compounds designated as SPG-1 to SPG-5. Isolation, structure elucidation of these compounds and results of biological studies are presented in Part-3 of the thesis.

**Section-3.1.2: Isolation and structure elucidation of SPG-1**

Compound SPG-1 was isolated as needle like crystals, mp 290°C from both the EtOAc and *n*-BuOH extracts of *Sarcochlamys pulcherrima* fruits by column chromatography. Its molecular formula was determined as C$_{30}$H$_{48}$O$_{3}$ from the molecular ion peak at *m/z* 456.36 [M]$^+$ as well as from its $^{13}$C-NMR spectral data. The structure of compound SPG-1 was established as betulinic acid. It is a known compound and reported for the first time from this plant.

![SPG-1](image)

**Section-3.1.3: Isolation and structure elucidation of SPG-2**

Compound SPG-2, is a pale white powder, mp 276°C, gave an indication of steroid on Liebermann-Burchard test. The molecular structure of the compound SPG-2 was established as stigmast-5,22-dien-3-O-β-D-glucopyranoside.
Section -3.1.4: Isolation and structure elucidation of SPG-4

SPG-4 was obtained as white crystals. All the spectroscopic data were very close to that of 3-oxo-olean-12-ene. Hence the structure was established as 3-oxo-olean-12-ene for SPG-4.

Section -3.1.5: Isolation and structure elucidation of SPG-5

The compound SPG-5 isolated as white crystalline solid from n-BuOH subfraction of aqueous MeOH extract of *Sarcochlamys pulcherrima* fruits by silica gel column chromatography having $R_f$: 0.584, EtOAc-MeOH (9.5:0.5), MP: 269°C, Soluble in MeOH. The structure was established as tormentic acid.
Section-3.2: Biological studies of *Sarcochlamys pulcherrima*

Different subfractions as well as some isolated pure compounds of *Sarcochlamys pulcherrima* were tested for antimicrobial activity using different gram positive and gram negative bacteria. Overall findings of the result are discussed here as follows.

Section-3.2.2: Antibacterial activities of *Sarcochlamys pulcherrima*

SPG-1 & SPG-2 isolated from *Sarcochlamys pulcherrima* were tested for antimicrobial activity using different gram positive and gram negative bacteria. Details of this study have been reported in the thesis.

Section-3.2.3: Antidiabetic activities of *Sarcochlamys pulcherrima*

In α–glucosidase inhibition assay, SPG-1 showed significant α–glucosidase inhibition (81.04%) at 500μg/ml and comparable to that of acarbose (standard inhibitor) which showed 82.62% inhibition of α–glucosidase activity at the same concentration.

Section-3.2.4: Antioxidant activities of *Sarcochlamys pulcherrima*

Details of this study have been reported in the thesis.

PART- 4: Materials and methods.

Details experimental methods of isolation, biological studies and characterization of some compounds that were not discussed in Part – 1 to Part – 3 have been reported in this part of the thesis.