

**CHEMICAL DIVERSITY AND BIOLOGICAL ACTIVITY OF ENDEMIC PLANTS OF
KUALA KENIAM, TAMAN NEGARA PAHANG.**

BY :

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4. Enhanced Research Title and Objectives

Original Title as Proposed:

CHEMICAL DIVERSITY AND BIOLOGICAL ACTIVITY OF ENDEMIC PLANTS OF KUALA KENIAM, TAMAN NEGARA PAHANG.

Improved/Enhanced Title:

No Changes

Original Objectives as Proposed:

1. To perform phytochemical screening for presence of alkaloids, flavonoids, quinones etc of the plants collected in Kuala Keniam, Taman Negara.
2. To perform various biological assays such as antioxidant, antimicrobial, antidiabetic and antimalarial tests on the crude extract of the plant samples.
3. To isolate and purify the chemical constituents of the several prioritized plant samples using various chromatographic techniques such as column chromatography, radial chromatography, preparative thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).
4. To identify the pure isolated chemical constituents using modern spectroscopic techniques: 1D and 2D Nuclear Magnetic Resonance (NMR), Mass Spectrometry, UV and IR.
5. To test the chemical constituents for various biological activities above if the quantity of the compounds isolated is sufficient.

Improved/Enhanced Objectives:

1. To perform phytochemical screening for presence of alkaloids and flavonoids of the plants collected in Kuala Keniam, Taman Negara.
2. To perform antioxidant, antimicrobial and antidiabetic tests on the crude extract of the plant samples.
3. To isolate and purify the chemical constituents of one prioritized plant samples using various chromatographic techniques such as column chromatography, radial chromatography, preparative thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).
4. To identify the pure isolated chemical constituents using modern spectroscopic techniques: 1D and 2D Nuclear Magnetic Resonance (NMR), Mass Spectrometry, UV and IR.

5.2 Enhanced Executive Summary

(Abstract of the research)

Tropical plants with diverse chemical complexities are undoubtedly the most important natural resource in the search for bioactive natural products with potential use in pharmaceutical applications and healthcare. Seventy-six plant species of interest from twenty-five families were collected during two phytochemical expeditions in Kuala Keniam, Pahang. The plants were from family Rubiaceae, Annonaceae, Lecythidaceae, Myristicaceae, Tiliaceae, Rosaceae, Moraceae, Ulmaceae, Menispermaceae, Eupobiaceae, Lauraceae, Ebenaceae, Verbanaceae, Myrsinaceae, Simaroubaceae, Leeaceae, Rutaceae, Melastomaceae, Sterculiaceae, Celastraceae, Commelinaceae, Burseraceae, Convolvulaceae, Guttiferae and Pandanaceae. The plants were screened on site for presence of alkaloids and flavonoids. Several plants with high content of alkaloids and flavonoids were found. Alkaloids and flavonoids are classes of compounds with many therapeutic activities such as antioxidant, antimicrobial, anticancer and others. Selected plants were assayed for biological activities. 143 extracts produced from various parts of the plants were evaluated for free radical scavenging and antibacterial activities. As a results, 12 extracts were found to strong free radical scavengers, 9 extracts were moderate while others exhibited weak or no activity. For antibacterial screening, a dosage of 100 µg of each plant extract was subjected to disc diffusion antibacterial assay using 12 bacteria strains. Inhibition zones of 10 to 15 mm are regarded as moderate activity where as inhibition zones of more than 15 are considered strong. Inhibition zones of 7-10 mm are considered weak. Two plants from genus *Knema*, which are *Knema Malayana* and *Knema glauca* was found to possess promising antibacterial activity. For antidiabetic properties, 47 extracts were assayed for α -amylase and α -glucosidase inhibitory activities. Four plant species demonstrated potent α -amylase inhibition. The plants were *Burkillantus malaccensis* (stem), *Horsfieldia polyspherula* (leaves and stem) *Labisia pumila* (leaves and root) and *Phyllanthus pulcher* (stem). The IC_{50} values of these extracts ranged from 1.2 to 2.8 µg ml⁻¹. Four plant species may be classified as strong α -glucosidase inhibitors with IC_{50} values ranging from 2.4 to 6.1 µg ml⁻¹. The plants were *Gironniera parvifolia* (stem), *Horsfieldia polyspherula* (leaves and stem), *Phyllanthus pulcher* (leaves and stem) and *Rothmannia schoemania* (stem). Extracts of *P. pulcher* (stem) and *H. polyspherula* (leaves and stem) showed potent inhibitory activity against both enzymes. These data suggest that these plant extracts are potentially useful in the development of new antidiabetic remedies. Detailed phytochemical studies on *Renellia elliptica* lead to the isolation of interesting molecules; 2-Formyl-3-hydroxy-9,10-anthroquinone and 1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone which may become a platform for development of various useful commercial healthcare and pharmaceutical products form our tropical plants.

5.3 Introduction

Tropical plants with diverse chemical complexities are undoubtedly the most important natural resource in the search for bioactive natural products with potential use in pharmaceutical applications and healthcare. Phytochemical investigations on plants used in traditional medicine are targeted towards isolation and discovery of biologically active compounds. Biologically active chemical constituents isolated from these plants used in traditional medicine may be a source of new medicinal agents.

Malaysia being located in tropical belt has been classified as one the mega biodiversity country. It is estimated that there about 10000 species of higher plants and about 2000 species of lower plants available in Peninsular Malaysia with approximately 16% of these claimed to be used for medicinal purposes. So, our rich tropical rain forests represent an enormous reservoir of chemically diverse molecules with potential therapeutic activity waiting to be discovered. Taman Negara Malaysia being a protected tropical forest basically is still unexploited and has great potential for discovery of important useful chemical compounds.

In the development of therapeutically useful natural products, phytochemical investigation of the endemic plants is a critical and essential step. In this work, a botanical survey followed by phytochemical and biological screening of plants endemic to Kuala Keniam, Taman Negara was conducted. Flowering plants were collected and documented. Parts of collected plants were screened on-site for presence of alkaloids and flavonoids. The plant materials were extracted using various organic solvents and the extracts subjected to a battery of available bioassays in order to prioritize the plant samples for further chemical investigations. Upon prioritization, a bigger scale (3 -5 kg) sample collection were conducted. The selected sample were then be chemically studied in detail. At this stage various chromatographic techniques were employed in order to isolate the chemical constituents. Identification of the isolated chemical constituents were performed using modern spectroscopic techniques. For biological testing, the plant materials were extracted using organic solvent and the extracts subjected to antioxidant assay, antimicrobial assay and α -amylase and α -glucosidase inhibitory activities. Discovery of potential biologically active compounds may lead to development of useful commercial natural healthcare products.