ISSN 2355-7079
E-ISSN 2406-8195
No. 754/AU3/P2MI-LIPI/08/2016
No: 21/E/KPT/2018

# Indonesian Journal of Forestry Research

Vol. 7 No. 2, October 2020

Ministry of Environment and Forestry
Research, Development and Innovation Agency
Indonesia

# Indonesian Journal of Forestry Research

Vol. 7 No. 2, October 2020

## ANNALS OF THE INDONESIAN JOURNAL OF FORESTRY RESEARCH

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IJFR is published by Research, Development and Innovation Agency (FOERDIA), Ministry of Environment and Forestry, formerly known as Forestry Research and Development Agency, the Ministry of Forestry Republic of Indonesia.
**ISSN print: 2355-7079**
**ISSN electronics: 2406-8195**
Electronic edition is available at:
**http://ejournal.forda-mof.org/ejournal-litbang/index.php/IJFR**

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# Indonesian Journal of Forestry Research

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# Indonesian Journal of Forestry Research

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## Acknowledgement

The Indonesian Journal of Forestry Research expresses sincere appreciation to all reviewers for selflessly contributing their expertise and time to the reviewing process, which is crucial to ensurethe quality and substantive impact of the journal. The journal's editors and authors are grateful for the reviewers' efforts in evaluating and assessing the articles submitted for of publication, regardless of the outcome (acceptance or rejection).

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THE GROWTH OF LOCAL TREE SPECIES ON POST-COAL MINING AREAS IN EAST KALIMANTAN

(*PERTUMBUHAN BEBERAPA JENIS POHON LOKAL DI LAHAN PASCA TAMBANG BATU BARA DI KALIMANTAN TIMUR*)

Wilayah pasca tambang batu bara perlu direhabilitasi untuk mengembalikan fungsinya. Tidak semua tanaman dapat tumbuh dengan baik di lahan bekas tambang batu bara karena intensitas cahaya yang berlebihan dan fluktuasi suhu yang ekstrim. Studi ini bertujuan untuk menentukan jenis pohon lokal yang cocok untuk merehabilitasi areal pasca tambang. Penanaman dilakukan pada bulan November 2012, dan pengamatan dilakukan pada bulan November 2015. Lokasi penelitian berada di Kabupaten Samboja, Kutai Kartanegara, Provinsi Kalimantan Timur, Indonesia. Hasil penelitian menunjukkan bahwa tujuh jenis pohon mampu bertahan hidup dengan baik di lahan bekas penambangan, yaitu: Vitex pinnata L., Syzygium scortechinii (Merr.) Merr. & Perry, Syzygium polyanthum (Wight) Walp., Shorea balangeran (Korth.) Burck, Macaranga motleyana (Mull.Arg.) Mull. Arg., Cleistanthus myrianthus (Hassk.) Kurz dan Syzygium lineatum (DC.) Merr. & L.M.Perry. Dari ketuju jenis tersebut, V. pinnata, Sy. scortechinii dan Sy. polyanthum menunjukkan persen hidup dan pertumbuhan terbaik. Studi ini menunjukkan bahwa ketiga jenis tersebut merupakan jenis pohon lokal yang sangat baik untuk rehabilitasi lahan pasca tambang batu bara, tidak hanya karena tingkat kelangsungan hidup mereka yang tinggi (≥80%) dan cepat tumbuh, tetapi juga menghasilkan buah dan bunga yang disukai satwa liar.

Kata kunci: Rehabilitasi, jenis pohon lokal, area pasca tambang batu bara

UDC/ODC 630*585(669)

John A. Ogbodo, Loretta M . Obimdike and Yason Benison

REMOTE SENSING FOR URBAN TREE CANOPY CHANGE DETECTION WITH LANDSAT SATELLITE DATA IN NNAMDI AZIKIWE UNIVERSITY, AWKA – NIGERIA

(*PENGINDERAAN JAUH UNTUK MENDETEKSI PERUBAHAN KANOPI POHON KAWASAN PERKOTAAN MENGGUNAKAN DATA SATELIT LANDSAT DI NNAMDI AZIKIWE UNIVERSITY, AWKA - NIGERIA*)

Kanopi pohon di kawasan perkotaan seperti kanopi pohon di perbatasan wilayah suatu universitas merupakan ukuran tutupan pohon wilayah universitas sebagai suatu persentase dari total luas lahan. Penelitian ini menganalisis perubahan spatio-temporal dari kanopi pohon perkotaan di wilayah Nnamdi Azikiwe University Awka -Nigeria. Data Landsat tahun 1991, 2001, 2011, dan 2019 dianalisis menggunakan *Maximum Likelihood Classifier* dan Confusion *Matrix Spatial Analyst* pada perangkat lunak ArcGIS 10.7.1. Dalam hal penurunan tutupan pohon, hasilnya menunjukkan penurunan yang stabil dari -31,59 ha antara tahun 1991 dan 2001; -82,32 ha (2001/2011) dan -64,53 ha (2011/2019). Pada tahun 1991, luasan awal seluas 9,4 ha pada tahun 1991, pembangunan infrastruktur fisik semakin meningkat seluas 16,92 ha antara tahun 1991 dan 2001; 43,79 ha 2001/2011 dan 12,37 ha antara tahun 2011 dan 2019. Pendorong utama perubahan tutupan pohon di wilayah studi terkait dengan perluasan infrastruktur fisik dan perladangan berpindah dari penduduk desa yang bertetangga, melakukan perambahan ke wilayah yang dianalisis. Dari penelitian dapat disimpulkan bahwa hutan tropis di kawasan kampus termasuk yang menghadapi banyak ancaman, seperti yang ditimbulkan oleh pembangunan infrastruktur fisik yang tidak diatur dan kurangnya investasi dan pengelolaan peninggalan hutan. Untuk itu, disarankan agar universitas-universitas di Nigeria berinvestasi dan melestarikan lanskap hutan yang ada untuk mempromosikan pengelolaan sumber daya lahan, mengikuti pencapaian strategi tujuan pembangunan berkelanjutan nomor 15 (SDG-15).

Kata kunci: Deteksi perubahan, koefisien Kappa, penginderaan jauh Landsat, Nnamdi Azikiwe University Awka, perluasan kota

UDC/ODC 630*232.315

Arif Irawan, Hanif N. Hidayah, Iwanuddin, Julianus Kinho, and Jafred E. Halawane

STORAGE TECHNIQUES OF CEMPAKA WASIAN (*Magnolia tsiampaca* (Miq.) Dandy) SEEDS

(*TEKNIK PENYIMPANAN BENIH CEMPAKA WASIAN (*Magnolia tsiampaca *(Miq.) Dandy)*.)

Cempaka wasian merupakan jenis kayu yang memiliki nilai historis sangat erat dengan budaya masyarakat Minahasa. Pengembangan hutan tanaman cempaka wasian perlu menjadi prioritas mengingat tingkat kebutuhannya cukup tinggi. Penyediaan benih dalam jumlah yang banyak dan pada waktu yang tepat sangat berkaitan dengan usaha metode penyimpanan benih yang sesuai. Penelitian ini bertujuan untuk mengetahui pengaruh waktu, tempat, dan wadah penyimpanan yang sesuai untuk benih cempaka wasian. Rancangan yang digunakan dalam penelitian ini adalah acak lengkap yang disusun dengan pola factorial yang terdiri dari 3 (tiga) faktor, yaitu: 1) waktu penyimpanan, 2) ruang penyimpanan, dan 3) wadah penyimpanan. Waktu penyimpanan terdiri dari 4 faktor, yaitu 2; 3; 4; dan 5 bulan, tempat penyimpanan terdiri dari 3 faktor yaitu ruang kulkas; ruang kamar; dan ruang AC. Sedangkan wadah penyimpanan terdiri dari kain blacu; aluminium foil; dan plastik. Hasil penelitian menunjukkan bahwa benih cempaka wasian masih dapat berkecambah optimal walaupun disimpan selama 5 (lima) bulan apabila menggunakan wadah simpan plastik dalam ruang kulkas.

Kata kunci: Benih, cempaka wasian, lama penyimpanan, wadah

UDC/ODC 630*414.2

Rizki Maharani, Andrian Fernandes, Maman Turjaman, Harlinda Kuspradini, and Ganis Lukmandaru

CHEMICAL AND ORGANOLEPTIC PROPERTIES OF BEKAI (*Pycnarrhena tumefacta* Miers) LEAVES FOR FLAVOURING AGENT (BIO-VETSIN)

(*KARAKTERISTIK KIMIA DAN ORGANOLEPTIK DAUN BEKAI (*Pycnarrhena tumefacta *Miers) UNTUK PENYEDAP ALAMI (BIO-VETSIN)*.)

Masakan "enak" cenderung menggunakan bahan penyedap kimia yang mengandung monosodium glutamat (MSG). Pemanfaatan MSG dalam jangka panjang dapat menyebabkan gangguan kesehatan, terutama pemicu sel kanker. Oleh karena itu perlu dilakukan pengenalan dan penambahan zat penyedap alami untuk mengatasi gangguan kesehatan tersebut, seperti daun bekai. Bekai (Pycnarrhena tumefacta *Miers*) dikenal sebagai penyedap alami (bio-vetsin) dalam masakan oleh masyarakat di Desa Nyapa Indah, Berau, Kalimantan Timur, Indonesia. Namun hingga saat ini belum ada dukungan analisis

yang tepat untuk pemanfaatan daun bekai secara luas. Penelitian ini menentukan keberadaan fitokimia, anti oksidan dan analisis GC MS dari ekstrak daun bekai, serta lima klasifikasi hedonik uji organoleptik, untuk memperkuat perlunya pemahaman yang lebih baik tentang reaksi konsumen dalam hal kemungkinan penerimaan penyedap asal daun bekai yang ditambahkan dalam sup sebagai bio-vetsin. Penelitian terbaru menunjukkan bahwa skrining kualitatif senyawa fitokimia pada ekstrak etanol daun bekai menunjukkan adanya alkaloid, flavonoid, tanin dan steroid. Anti oksidan daun bekai dengan metode 2,2-difenil-1-pikrilhidrol (DPPH) menunjukkan ekstrak pekat memiliki kandungan 80,1% yang diperkirakan dapat meningkatkan kekebalan tubuh untuk menghambat aksi sel kanker. Analisis GC MS diduga ekstrak daun bekai mengandung lima senyawa utama yaitu oksiran dodesil, gamma sitosterol, vitamin E (α tokoferol), 9.12-oktadekadienoat (Z, Z) - (asam linoleat alami), dan 3-Tetradekanynoat (asam miristat). Senyawa kimia yang terkait dengan fitokimianya diduga mengandung aktivitas antioksidan yang kuat dan beberapa di antaranya biasa digunakan sebagai agen penyedap masakan pada beberapa industri makanan. Sedangkan dari hasil uji organoleptik keberadaan tiga varian soto menunjukkan bahwa dengan tambahan daun bekai paling diterima persepsi anak-anak karena memiliki bau yang khas, gurih dan tidak berubah warna dibandingkan dengan penambahan MSG dan kontrol. Dengan demikian daun bekai dapat digunakan sebagai inovasi untuk makanan sehat dan peluang pasar baru untuk pengganti MSG.

Kata kunci: Daun bekai, phytochemical, anti-oksidan, GC-MS, penyedap alami, organoleptik

UDC/ODC 630*19(540)

Rabishankar Sengupta, and Sudhansu S. Dash

A COMPREHENSIVE INVENTORY AND ECOLOGICAL ASSESSMENT OF ALIEN PLANT INVASION IN MIZORAM, INDIA

(*INVENTARISASI KOMPREHENSIF DAN PENILAIAN EKOLOGI INVASI JENIS-JENIS ASING DI MIZORAM, INDIA*)

Invasi oleh tumbuhan asing memiliki efek yang merugikan pada komunitas tumbuhan hutan alam, yang menyebabkan hilangnya spesies asli. Inventarisasi tumbuhan asing memudahkan untuk menentukan potensi ancaman terhadap keanekaragaman hayati tumbuhan alami. Tulisan ini mengevaluasi tumbuhan asing di Mizoram (wilayah hotspot Indo-Burma) dan merupakan inventarisasi otentik pertama tanaman asing Mizoram bersama dengan keanekaragaman, aspek ekologi, asal-usul dan status invasi mereka. Survei lapangan ekstensif dilakukan dari Juli 2018 hingga September 2019 di berbagai kawasan lindung Mizoram. Pengambilan sampel mengadopsi Teknik Pengambilan Sampel Acak menggunakan kuadrat bersarang di plot berukuran 400 $m^2$ (20 m x 20 m), di mana ukuran kuadrat 5 m x 5 m diletakkan untuk semak dan 1 m x 1 m untuk tumbuhan. Spesimen tanaman dikumpulkan, dikeringkan, diracun dengan 0,1% Mercuric Chloride ($MgCl_2$); voucher spesimen disiapkan dan disimpan di Central National Herbarium, Botanical Survey of India (CAL) setelah identifikasi yang tepat. Hasil penelitian menunjukkan terjadinya invasi 163 spesies tumbuhan asing di bawah 135 genera milik 51 famili di Mizoram. Spesies terbesar yang teridentifikasi adalah famili Asteraceae dengan 29 spesies diikuti oleh Leguminosae (19 spesies), Convolvulaceae (9 spesies), Euphorbiaceae (7 spesies) & Amaranthaceae (7 spesies). 57,66% spesies asing yang dilaporkan dari Mizoram berasal dari Amerika diikuti oleh 11,65% dari Afrika, 7,36% dari Meksiko & 5,52% dari Australia. Analisis bentuk kehidupan menunjukkan keberadaan 58,64% tumbuhan, 15,43% pohon dan 9,87% semak. Dari seluruh tumbuhan asing yang tercatat, 91 jenis dimanfaatkan untuk pengobatan tradisional, 43 jenis sebagai tanaman hias, 15 jenis untuk dimakan, sembilan jenis digunakan sebagai kayu dan empat jenis digunakan sebagai pupuk hijau. Studi tersebut juga merekomendasikan sepuluh spesies paling membahayakan, lima tanaman neo-invasif, yang memiliki potensi ancaman terbesar bagi flora asli. Proses dan kemungkinan penyebab invasi di negara bagian juga dibahas secara singkat, yang dapat digunakan dalam penyusunan kebijakan konservasi atau pengelolaan hutan.

Kata kunci: Tumbuhan asing, invasif, neo-invasif, keanekaragaman hayati, Mizoram

UDC/ODC 630*892.52

Gunawan Pasaribu, Totok K. Waluyo, Gustan Pari, and Novitri Hastuti

THE EFFECTIVENESS OF GLUCOMANNAN AND NANO ACTIVATED-CARBON AS HYPERCHOLESTEROL-LOWERING AGENTS

(*EFEKTIVITAS GLUKOMANAN DAN NANO KARBON AKTIF SEBAGAI AGEN PENURUN KOLESTEROL*)

Tanaman porang (konjak) telah lama digunakan sebagai sumber makanan dan obat tradisional. Glukomanan yang berasal dari tanaman tersebut telah digunakan untuk berbagai kegunaan sebagai antidiabetes dan juga sebagai antihiperkolesterol. Tujuan dari penelitian ini adalah untuk mengujir aktivitas kolesterol pada tikus melalui perlakuan kombinasi tepung porang dan nano karbon aktif. Kombinasi porang dan nano karbon aktif diuji pada tikus jantan *strain* Sprague Dawley untuk menguji aktivitas antihiperkolesterolemia. Hasilnya menunjukkan bahwa aktivitas antikolesterol porang dan nano karbon aktif menunjukkan penurunan tingkat kolesterol dalam darah tikus. Namun, perlakuan yang berbeda antara porang tanpa pencucian dan porang dengan pencucian dengan nano karbon aktif menunjukkan bahwa penurunan kadar kolesterol sedikit berbeda (16–18%). Kandungan glukomanan yang rendah dianggap cukup efektif dalam menurunkan kadar kolesterol dalam darah tikus, dibandingkan dengan kontrol positif (simvastatin); keduanya mencapai penurunan 18%; sehingga menunjukkan potensi pemanfaatannya sebagai makanan fungsional untuk menurunkan kolesterol. Pengaruh nano karbon aktif dalam meningkatkan aktivitas antihiperkolesterolemia (tepung porang yang dicuci) ternyata tidak banyak membantu dalam meningkatkan penurunan kadar kolesterol dalam darah tikus. Glukomanan dalam tepung porang yang dicuci dan tepung porang yang dicuci + nano karbon aktif menunjukkan potensi pemanfaatannya sebagai agen antihiperkolesterol. Namun, porang yang tidak dicuci berpotensi sebagai makanan fungsional untuk menurunkan kolesterol.

Kata kunci: Porang, antihiperkolesterol, nano karbon aktif, glukomanan

# THE GROWTH OF LOCAL TREE SPECIES ON POST-COAL MINING AREAS IN EAST KALIMANTAN

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Received: 2 June 2020, Revised: 1 October 2020, Accepted: 1 October 2020

THE GROWTH OF LOCAL TREE SPECIES ON POST-COAL MINING AREAS IN EAST KALIMANTAN. Post-coal mining areas need rehabilitation to restore its functionality. Not all plants could grow well on bare ex-coal mining area because of the excessive light intensity and extreme temperature fluctuations. This study is aimed to determine suitable local tree species for rehabilitating mined areas. Planting was carried out in November 2012, and observations were made in November 2015. The study site was in the district of Samboja, Kutai Kartanegara, East Kalimantan Province, Indonesia. Research results revealed that seven tree species survived well in the ex-coal mining land, i.e., *Vitex pinnata* L., *Syzygium scortechinii* (Merr.) Merr. & Perry, *Syzygium polyanthum* (Wight) Walp., *Shorea balangeran* (Korth.) Burck, *Macaranga motleyana* (Mull.Arg.) Mull.Arg., *Cleistanthus myrianthus* (Hassk.) Kurz and *Syzygium lineatum* (DC.) Merr. & L.M. Perry. From the seven species *V. pinnata*, *S. scortechinii* and *S. polyanthum* performed best in both survival and growth rates. This study suggests those three species are excellent local tree species for ex-coal mining rehabilitation, not only because of their high survival rate (≥80%) and fast-growing but also they produce favourable fruits and flowers for wildlife.

Keywords: Rehabilitation, local tree species, post-coal mining area

*PERTUMBUHAN BEBERAPA JENIS POHON LOKAL DI LAHAN PASCA TAMBANG BATU BARA DI KALIMANTAN TIMUR. Daerah bekas tambang batu bara perlu rehabilitasi untuk mengembalikan fungsinya. Namun, tidak semua jenis tanaman dapat tumbuh dengan baik di lahan bekas tambang batu bara, karena kawasan yang terbuka menjadikan intensitas cahaya matahari berlebihan dan fluktuasi suhu yang ekstrim. Penelitian ini bertujuan untuk menentukan jenis pohon lokal yang cocok untuk merehabilitasi areal bekas tambang. Penanaman dilakukan pada bulan November 2012, dan observasi dilakukan pada bulan November 2015. Lokasi penelitian berada di Kabupaten Samboja, Kutai Kartanegara, Provinsi Kalimantan Timur. Hasil penelitian menunjukkan bahwa terdapat tujuh jenis pohon yang mampu bertahan hidup dengan baik di lahan bekas tambang batubara, yaitu* Vitex pinnata *L.,* Syzygium scortechinii *(Merr.) Merr. & Perry,* Syzygium polyanthum *(Wight) Walp.,* Shorea balangeran *(Korth.) Burck,* Macaranga motleyana *(Mull.Arg.) Mull.Arg.,* Cleistanthus myrianthus *(Hassk.) Kurz dan* Syzygium lineatum *(DC.) Merr. & L.M.Perry. Dari tujuh spesies tersebut,* V. pinnata, S. scortechinii *dan* S. polyanthum *tercatat memiliki pertumbuhan terbaik dan mampu bertahan dalam lahan terbuka. Studi ini menunjukkan bahwa ketiga spesies tersebut adalah spesies pohon lokal yang disarankan untuk merehabilitasi tambang batu bara, tidak hanya karena tingkat hidupnya tinggi (≥80%) dan cepat tumbuh, namun juga menghasilkan buah dan bunga yang menguntungkan bagi satwa liar.*

*Kata kunci: Rehabilitasi, jenis pohon lokal, area pasca tambang batu bara*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.83-97 

## I. INTRODUCTION

In the National Energy Conservation Master Plan, it is mentioned that coal is promoted to be one of the primary energy sources in Indonesia by 2020 including East Kalimantan Province (Kusmana, Setiadi, & Al-Anshary, 2013). Coal is one of the primary energy sources to generate electricity, which is essential to support economic growth for East Kalimantan and also for the whole country. The contribution of mining and quarrying sector to the economy in this province is estimated to increase from 36.2% to 50.5% from 2001 to 2011(Ishak, 2013). However, coal mining has resulted in negative environmental impacts such as loss of biodiversity (Pandey, Agrawal, & Singh, 2014), degradation of the watershed quality and quantity (Merem et al., 2014; Zegre, Miller, Maxwell, & Lamont, 2014), as well as toxicity in the aquatic environment (Turner et al., 2013). Therefore, sustainable coal mining practices in East Kalimantan need to be promoted by reducing its negative impacts on the environment.

Reclamation and rehabilitation of post-coal mining areas need to be conducted to reduce the negative impacts of coal mining activities on the environment. These activities ensure ecosystem services to recover to the previous functions, which are essential to support human well-being and prevent further negative impacts on the ground (Yassir, 2013). Furthermore, based on the national regulation Act No 4/ 2009 about Mineral and Coal Mining in Indonesia, rehabilitation and reclamation after coal extractions are an obligation for the mining concession holders in this country. Therefore, reclamation and restoration are an essential activity to support sustainable mining in Indonesia (Mansur, 2010).

In the coal mining area rehabilitation projects, the use of local tree species in the species selection is one of the key factors to the success of the project. The local tree species have several advantages, such as more adaptive to the environment, maintaining the genetic integrity of the population of local species, preventing the invasion of exotic or non-local species (Gray, 2002). Besides, these local plant species could also contribute to the conservation programs. Unlike exotic plant species, the use of local plant species has minimum impacts in altering the species composition and structure of the landscape from the previous state before mining activities were conducted. On the other hand, the use of exotic plant species has some negative impacts on the environment and conservation programs (Radiansyah et al., 2015).

The success of revegetation can be evaluated by determining the plant's growth performance (Istomo, Setiadi, & Putri, 2013). Growth performance, which can be described as plant's height, diameter and survival rate, is an important aspect to observe in this study because it can be the indicators for plant species evaluation in adapting to a new environment. Environmental conditions in the post-coal mining area are different compared to the native habitat of the ten local plant species in this study. A study conducted by Villacís, Casanoves, Hang, Keesstra, and Armas (2016) by measuring height, tree diameter, and survival rate as growth parameter for 20 plant species in the rehabilitation of post-oil mining areas in Amazon Basin. That study aimed to provide a list of plant species suitable for post-oil mining rehabilitation. Meanwhile, a study conducted by Todd, Rufaut, Craw, and Begbie (2009) used plant survival and plant height growth in an opencast coal mining rehabilitation using indigenous plant species in New Zealand. Besides, Mushia, Ramoelo, and Ayisi (2016) used plant height, plant cover and plant biomass as growth indicators in a study determining the impacts of coal mine stockpile quality on plant growth and productivity.

In 2012, a rehabilitation project of the post-coal mining area had been conducted by planting ten local tree species in East Kalimantan, Indonesia (Adman & Yassir, 2016). The study reported that three tree species had a survival rate of more than 90% at the age of one year, indicating that they can grow well on post-coal mining areas. Those species are *Vitex pinnata* L., *Syzygium scortechinii* (King) P. Chanaranothai & J. Parnell, Kew, *Syzygium polyanthum* (Wight)

Walp. It is also reported that although they had a low survival rate, *Bridelia glauca* Blume and *Ficus variegata* Blume, could again grow well on post-coal mining land during the first year. According to a study, the critical period occurred between one to two years after planting for plant establishment in the tropical areas (Villacís et al., 2016). Therefore, this study aimed to evaluate the further performance of the ten local tree species planted in post-coal mining areas in East Kalimantan. This paper reported the growth performance of the ten local tree species in the post-coal mining area at the age of 4 years. It is crucial to provide alternative local plant species that are suitable to the local environment, which is also essential for conservation programs rather than exotic species.

## II. MATERIAL AND METHOD

### A. Study Site and Materials

The study area was a post-coal mining land at S01°00'44.1" E116°54'27.2", located in the district of Samboja, Kutai Kartanegara, East Kalimantan Province, Indonesia. The study was carried out in November 2012 by planting ten local tree species in one of the company's post-mining areas. The selection of the ten species (Tabel 1) was based on recommendations of the study reported by Yassir and Omon (2009).

### B. Methods

Seedlings (wildlings) of the ten local plant species were collected from the forests located around the mining areas. After collection, the seedlings were transported and maintained in the nursery under 50% shading. Pot size used was 10 x 15 cm filled with a mixture of topsoil and compost with a ratio of 1: 1 (v/v). During the first three months, seedlings were kept under a lid. The lid was used to keep the humidity high (>80%). Watering inside the lid was done at humidity less than 80%. After the seedlings produced new shoots, the lid was opened gradually starting from 10% opening for the 1st week, then 25% at the following week, then 50%, 75% to 100% opening, respectively with a one-week interval. After the lid opened 100%, the seedlings were kept in the nursery until they were ready for planting (± 5-7 months). Watering in the nursery was done twice a day, if it did not rain, to maintain humidity, and it was reduced if it is raining.

The seedlings were also sorted to obtain a uniform size in diameter and height before planting. Meanwhile, the planting area was covered by topsoil with ± 50 cm thickness based

Table 1. The botanical name of local plant species planted in this study

| No | Botanical name | Family | Local name | Uses |
|---|---|---|---|---|
| 1. | *Vitex pinnata* L. | Lamiaceae | Laban | Wood construction, firewood, medicinal plant |
| 2. | *Syzygium polyanthum* (Wight) Walp | Myrtaceae | Salam | Flavouring spices, medicinal plant |
| 3. | *Cleistanthus myrianthus* (Hassk.) Kurz | Phyllanthaceae | Jari-jari | - |
| 4. | *Syzygium lineatum* (Blume) Merr. & Perry | Myrtaceae | Gelam tikus | Wood construction, medicinal plant, fruits edible |
| 5. | *Syzygium scortechinii* (Merr.) Merr. & Perry | Myrtaceae | Obah air | Fruits edible |
| 6. | *Bridelia glauca* Blume | Phyllanthaceae | Kanidei | Wood construction, fruits edible |
| 7. | *Ficus variegata* Blume | Moraceae | Nyawai | Wood construction, fruits edible |
| 8. | *Schima wallichii* (DC) Korth. | Theaceae | Puspa | Plywood, pulp for paper, medicinal plant |
| 9. | *Macaranga motleyana* (Müll. Arg.) Müll. Arg. | Euphorbiaceae | Mahang | - |
| 10. | *Shorea balangeran* (Korth.) Burck | Dipterocarpaceae | Kahoi | Wood construction. |

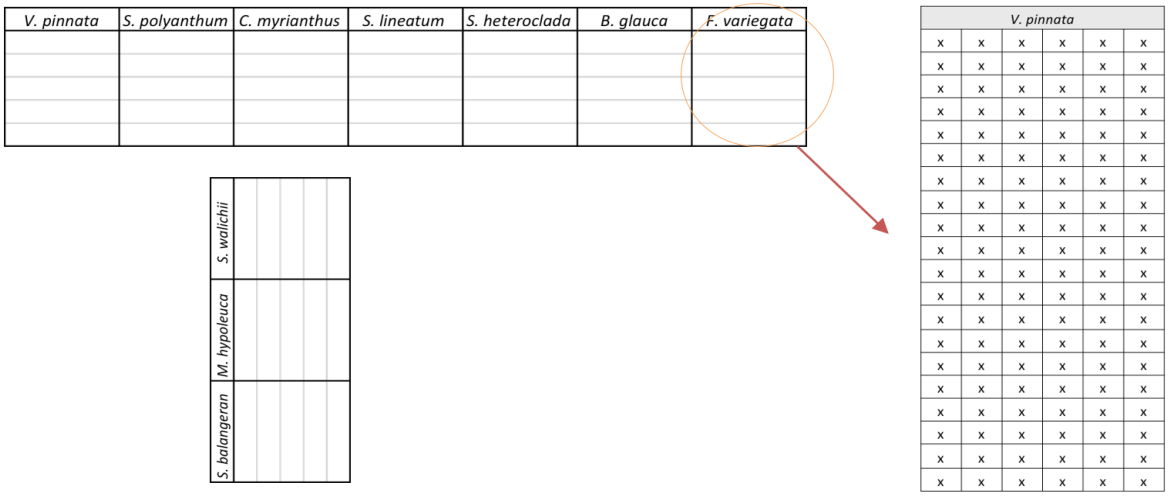


Figure 1. Planting layout and research design

on the company's standard operating procedure before planting. The size of the planting holes was 30 cm in length, width and depth. Each of the planting holes was filled with ± 1 kg compost and mixed with the soil for fertilization of the plants. The compost was made from decomposed remains of plants. The compost was applied once before planting. Each plant species was planted in six lines, consisting of 20 plants in each line, with 3 m x 3 m spacing (Figure 1). Therefore, 1200 plants were planted in the area, and the dead plants were not re-planted. This layout was implemented due to the availability of the post-coal mining area in the company. After planting, weeding was also regularly conducted four times a year to eliminate weeds and providing growing space for the plants. Planting holes, compost dosage and plant spacing, were based on standard operating procedures applied by PT. Singlurus Pratama.

Observations on the growth of the ten local plant species were regularly conducted every six months up to the age of four year. Data collection included plants' survival rates, heights and diameters at planting and after four years after planting and soil chemical characteristics before and after three years of planting. Survival rate was indicated by the percentage of plants that survived divided by the total number of planted plants. Plant's height was consistently measured from the ground level to the highest top of the plant, while the diameter was measured at ± 10 cm above ground level.

### C. Analysis

ANOVA (analysis of variance) was performed to identify species performances among the ten local plant species. It was followed by Tukey HSD test if the analysis of variance showed a significant difference between variables. The ANOVA result tables will not be shown, but the results of the Tukey HSD Test will be shown in histograms, and the statistical difference will be distinguished by letters. Before the analysis of variance, the data were tested to identify the normality, and if the data were not distributed normally, then they will be transformed using logarithms. Beside ANOVA, the height and diameter growth data are also depicted by using a graphic to observe the growing patterns.

## III. RESULT AND DISCUSSION

### A. Plant Survival Rates of the 10 Local Tree Species in the Post-Coal Mining Land

The results showed that seven of the ten local plant species planted on the post-coal mining area had survival rates above 80% at the age of four years. Among seven species, three of them had a survival rate above 90%, i.e. *S. polyanthum*,

*S. scortechinii* and *V. pinnata* (Figure 2). Survival rate indicates the number of surviving plants in the research site, including plants that grow slowly or it is in static growth. There were also three species having survival rates below 70%; i.e. *F. variegata*, *B. glauca* and *S. wallichii*, in which *F. variegata* had the lowest value, 32%.

In the first observation year, these three plant species also had low survival rates compared to the other seven local plants planted on the research site (Adman & Yassir, 2016). However, in general, plant death had occurred to almost all species of plants observed from 1 year to 4 years. It is indicated by a decrease in the plant's survival rate, except for *V. pinnata*, which showed consistently 100% survival rate. This means that death has occurred to the nine species of plants at a different rate. The decrease in the survival rate of plants indicates that some species were unable to adapt to the extreme mining environment at a different level. The reduction in survival rate from the 1 to 4 year varied between the ten local plant species. Five species have a decrease rate of less than 10%, i.e. *S. lineatum* (1.67%), *S. scortechinii* (2.50%), *S. wallichii* (3.34%), *C. myrianthus* (4.17%) and *S. polyanthum* (6.66%). The other species have a decrease rate below 20%, i.e. *S. balangeran* (13.33%), *M. motleyana* (15.00%) and *B. glauca* (16.67%). Meanwhile, *F. variegata* has the highest decrease rate of 36.50%.

Analysis of variance on the survival rate of the ten local tree species showed that the survival rate between species was significantly different. The result of multiple range tests and the details of plant survival rates are presented in Figure 2.

## B. The Average Height and Diameter Growth of the Ten Local Tree Species in the Post-Coal Mining Land

From ANOVA, it can be said that there were significant differences in terms of height and diameter growth rate among the ten local plant species tested in this study, as indicated in the results of multiple range tests (Figures 3 and 4). From the figures, several groups can be categorized by the plant growth in terms of height or diameter. The first group was *V. pinnata*, which had the most significant growth both in height and diameter compared to the other nine plant species during the four years. It grew by almost 5 m in height and 7.22 cm in diameter. Meanwhile, *S. polyanthum*, *S. balangeran* and *S. scortechinii* can be categorized as the second most impressive species in terms of height growth, with about 3 m growth in the four years. The third group of growth in terms

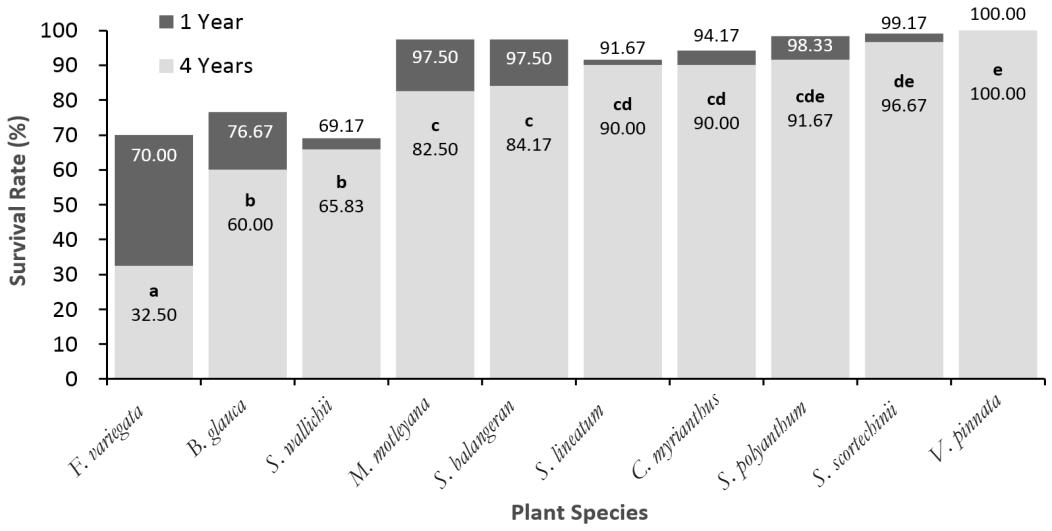


Figure 2. The survival rates of ten local tree species at years 1 and 4 in post-coal mining land, PT SGP (The same letters are not significantly different at 95% confidence level in Tukey HSD Test)

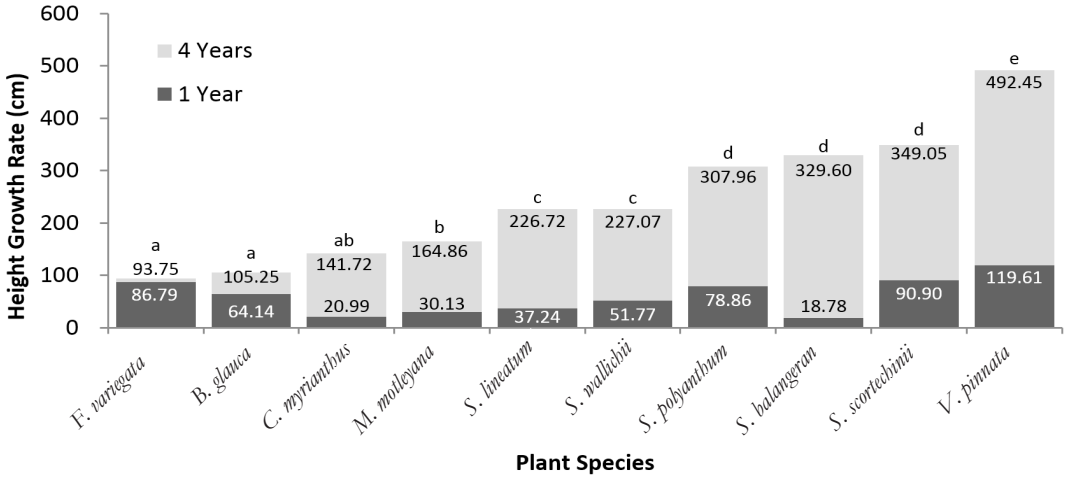


Figure 3. The height growth of ten local tree species at four years in post-coal mining land, PT SGP (The same letters are not significantly different at 95% confidence level in Tukey HSD Test)

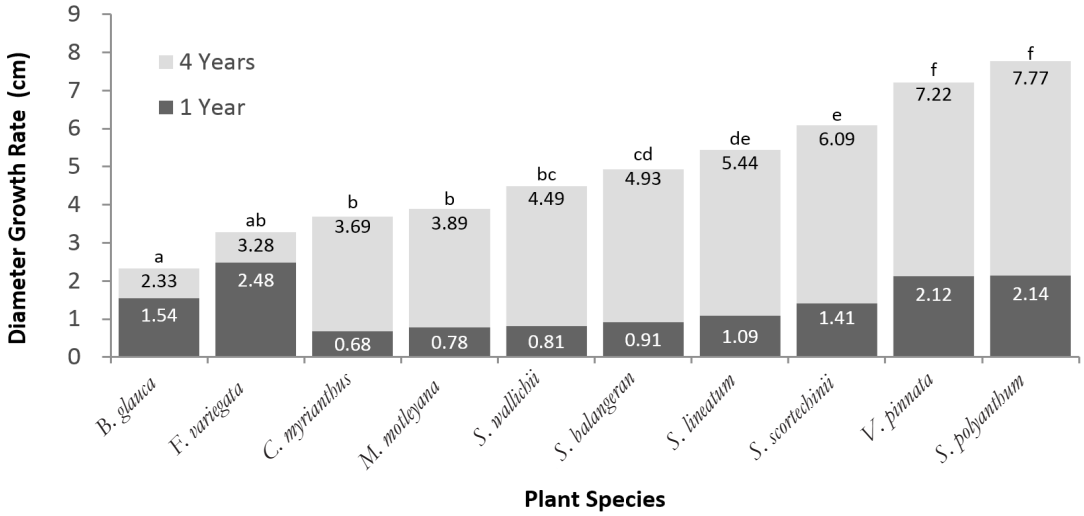


Figure 4. The diameter growth of ten local tree species at four years in post-coal mining land, PT SGP (The same letters are not significantly different at 95% confidence level in Tukey HSD Test)

of height was *S. lineatum* and *S. wallichii*, then followed by *C. myrianthus* and *M. motleyana* as the fourth group.

In terms of diameter, *S. polyanthum* can be categorized as the most impressive because its growth is close to *V. pinnata*, growing by about 7 cm during the four years. Meanwhile, *S. balangeran*, *S. lineatum* and *S. scortechinii* can be placed in the same group, with diameter growth range from 4.9 to 6 cm in the same period. The next group is *C. myrianthus*, *M. motleyana* and *S. wallichii*, with diameter growth from 3.5 to 4.5 cm during the same period. The last group is *F. variegata* and *B. glauca*, which can be considered to have the slowest growth, in terms of height and diameter compared to the other species.

## C. The Growth Pattern of the Ten Local Plant Species in the Post-Coal Mining Land

The growth pattern of ten local plant species is presented in Figures 5 and 6. From the figures, in general, all species observed in this study

experienced slow growth in terms of height and diameter in the first year after planting. In the following years, there was an acceleration in both height and diameter growth at various rates of almost all plant species observed. If we study the growth pattern in more detail, the growth acceleration occurred from month 12th to 30th. In the subsequent months, particularly from months 30th to 36th, the height and diameter growth was slower than in the previous period. Finally, in the subsequent period, from months 36th to 48th, the height and diameter growth began to accelerate, except for *B. glauca*.

In Figures 5 and 6, *V. pinnata* had shown a consistent and significant growth pattern in both diameter and height compared to the other plant species. Furthermore, although the diameter growth began to de-accelerate at the 30th months, this species' height growth is started to re-accelerate again in the 36th month. Meanwhile, *S. balangeran*'s height growth shows different pattern compared to the other species, after experiencing rapid growth during the first 30 months period, the height growth stagnated instead of growing from month 30th to 36th. This is because of a significant number of *S. balangeran* were experiencing 'partial death' during that time; therefore, the heights were measured at the new part of the plants that just grew from month 30th to month 48th. Partial death means that part of the total height of the plant is dead, which is followed by the growth of several new shoots in the plants in the subsequent period. On the other hand, *B. glauca* and *F. variegata* show different height growth pattern. After a slow growth in the first two years, they experienced stagnation in height growth from 24th month to 48th month, which was also caused by partial death in some plants.

In Figure 6, *S. polyanthum*, *V. pinnata*, and *S. scortechinii* have similar diameter growth pattern, showing the most significant and consistent growth up to the first 30th month and slightly decreasing in the subsequent 18 months, compared to the other species. Meanwhile, *B. glauca* and *F. variegata* show relatively similar diameter growth pattern with their height growth pattern. The slow diameter growth of the two species has occurred up to month 30th. While *F. variegata* experienced a slight increase in the diameter growth from month 30th to 36th followed by a continued stagnation (*the tree cannot grow negatively*) up to month 48th, *B. glauca* had experienced a steady decrease in diameter growth since month 30th.

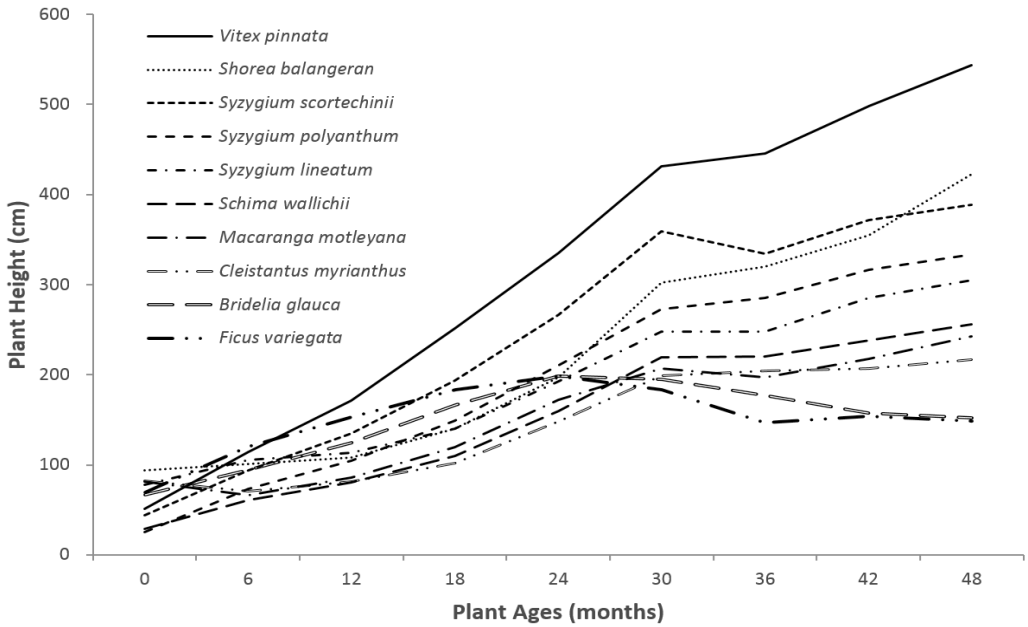


Figure 5. Height growth trends of ten local tree species after four years planted in post-coal mining land, PT SGP

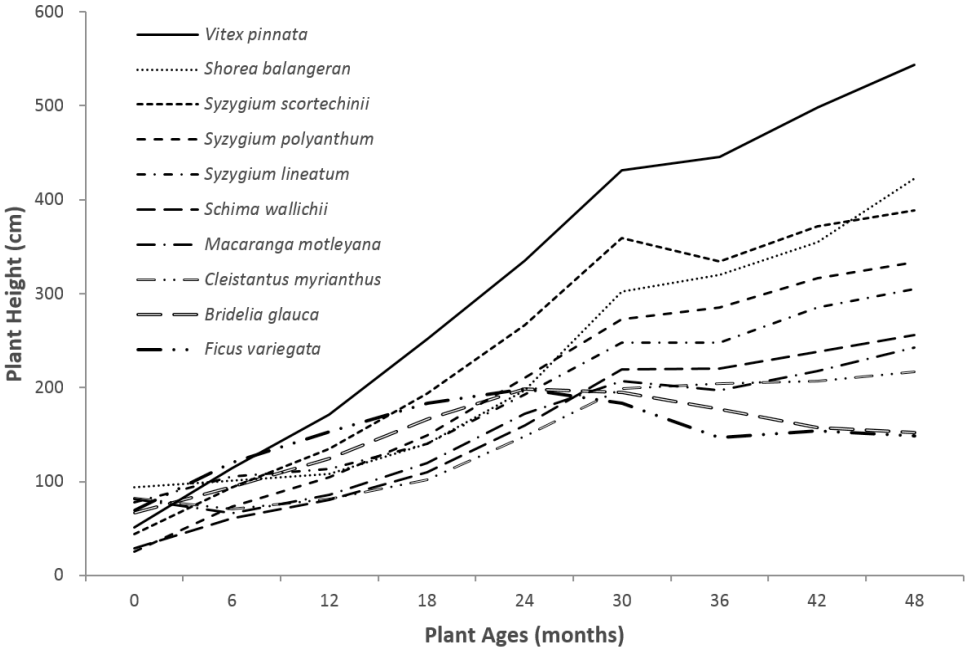


Figure 6. Diameter growth trends of ten local tree species after four years planted in post-coal mining land, PT SGP

## D. Plant Adaptation to the Environment of Rehabilitation Area

The main cause of the slow growth of almost all species of plants in the first year was due to the adaptation process from the nursery to the rehabilitation area. There were dramatic environmental changes from the nursery, in which the plants were kept before planted into the mining area, which is characterized by an open area, water-shortage, extremely high temperature, and soil compaction. On the other hand, in the nursery, the plants were controlled in a relatively cool temperature and well maintained. Furthermore, there is four typologies of soil degradation; highly degraded lands, moderately degraded lands, slightly degraded lands and improving lands (FAO, 2011). The severity of soil degradation in the post-mining areas can be categorized as highly degraded; in this type of degradation, the intervention option needed is rehabilitation. As a result, some plant species might need considerable time to adapt to the rehabilitation area.

Stressful environmental condition like water shortage in the post-coal mining areas during the first year of rehabilitation might result in a decrease in the amount of chlorophyll in the leaves of the plants (Ramírez et al., 2014). Death can occur to the plants; simply, they did not survive. However, the survived plants would re-sprout and re-grow. Nevertheless, some of the local tree species planted could re-sprout, but each of the plant species had different times to re-sprout and re-grow. Therefore, the ability to re-sprout is important for plants to survive and grow in post-coal mining land as a form of adaptation to extreme environmental conditions.

The other abiotic stress in the post-coal mining rehabilitation areas is the high temperature. A review reported that heat stress has some negative impacts on plants, depending on species types, genotype and heat duration (Bita & Gerats, 2013). From the review, there are several visible impacts to the plants resulting from high temperature; burned or senescence leaves and stems, damage of fruit and suppressing root growth leading to plant productivity loss. The review also reveals that heat stress can change the respiration and photosynthesis of the plants, causing a

decrease in plant productivity and shortened life cycle. It is also shown that a 5ºC increase in temperature above the normal condition can lead to a reduction in protein synthesis in the cellular system. Besides, heat stress also has some adverse impact on the molecular level of plants affecting the photosynthesis (Allakhverdiev et al., 2008). In the ecosystem, heat stress can cause tree mortality in the forest ecosystem (Anderegg, Kane, & Anderegg, 2013). Therefore, the slow growth of the ten local species in the first year after planting was caused by high temperature.

Although the analysis of the physical properties of the soil was not done, the issue of soil compaction is also one of the main features that potentially inhibiting plant growth. A review reported that severe soil compaction could result in stunted growth of shoot, root deformation and high mortality rate (Nawaz, Bourrié, & Trolard, 2013). From the review, it was also revealed that soil compaction also reduced the soil biodiversity; reducing the number of soil fauna and flora, enzymatic activity and microbial biomass. Moreover, a study reported that there were significant differences in terms of soil microbial communities between soil from post-mining areas and undisturbed soil, in which the controlled soil areas had higher microbial diversity compared to the soil of post-mining regions (de Quadros et al., 2016). However, another research showed that there was no difference in terms of growth and survival rates between trees planted in compacted soil and un-compacted soil in the post-mining areas in West Virginia, the USA (Emerson, Skousen, & Ziemkiewicz, 2009). This might be due to the age of seedlings planted in the study, which was two years old. Meanwhile, in this study, the age of the seedlings when they were planted was unknown because the seedlings were obtained from the natural forests. Thus, the compacted soil in this study might have a negative impact on plant growth, resulting in slow diameter and height development in the first year.

The analysis of soil chemical properties (Table 2) shows that among species, the pH values were generally very acid and decreased from 3.83–4.57 to 3.38–4.06. The Cation Exchange Capacities (CEC) varies, some have increased, and some dropped, although all are still in the low category. The N total increased under several species from very low to the standard type; otherwise, the C/N ratio generally decreased. The concentration of $P_2O_5$ and $K_2O$ were growing, especially for $K_2O$ that significantly increased, from low-medium to high-very high.

### E. Plant Growth

During four years, *V. pinnata* had a remarkable growth performance, indicated by reasonable survival rate, diameter and height growth, compared to the other nine species. This species also had relatively better adaptation to the environment in the early

Table 2. Local plant species planted in this study

| No | Species | pH | | CEC (meq./100 gr) | | N Total (%) | | C/N ratio | | P2O5 (ppm) | | K2O (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 Year | 3 Years | 0 Year | 3 Years | 0 Year | 3 Years | 0 Year | 3 Years | 0 Year | 3 Years | 0 Year | 3 Years |
| 1. | *Vitex pinnata* | 3.83[1)] | 3.38[1)] | 5.62[b)] | 9.79[b)] | 0.04[a)] | 0.11[b)] | 20.0[d)] | 8.07[b)] | 0.86[a)] | 5.71[a)] | 14.39[b)] | 71.24[e)] |
| 2. | *Syzygium polyanthum* | 4.34[1)] | 3.60[1)] | 7.84[b)] | 7.87[b)] | 0.06[a)] | 0.10[b)] | 14.9[c)] | 8.76[b)] | 0.94[a)] | 1.30[a)] | 19.63[b)] | 49.59[d)] |
| 3. | *Cleistanthus myrianthus* | 4.08[1)] | 4.06[1)] | 7.35[b)] | 7.70[b)] | 0.03[a)] | 0.10[b)] | 33.1[e)] | 8.30[b)] | 0.43[a)] | 5.71[a)] | 14.59[b)] | 57.99[d)] |
| 4. | *Syzygium lineatum* | 4.38[1)] | 3.82[1)] | 6.16[b)] | 7.41[b)] | 0.06[a)] | 0.11[b)] | 14.0[c)] | 12.04[c)] | 0.77[a)] | 2.47[a)] | 8.18[a)] | 57.67[d)] |
| 5. | *Syzygium scortechinii* | 4.54[1)] | 3.68[1)] | 7.45[b)] | 8.83[b)] | 0.06[a)] | 0.08[a)] | 14.2[c)] | 11.58[c)] | 0.94[a)] | 2.47[a)] | 11.01[b)] | 47.98[d)] |
| 6. | *Bridelia glauca* | 4.57[2)] | 3.92[1)] | 7.06[b)] | 6.70 [b)] | 0.06[a)] | 0.05[a)] | 15.3[c)] | 15.68[c)] | 1.29[a)] | 1.30[a)] | 12.66[b)] | 37.96[c)] |
| 7. | *Ficus variegata* | 3.87[1)] | 3.86[1)] | 7.08[b)] | 6.93[b)] | 0.05[a)] | 0.10[b)] | 17.5[d)] | 7.84[b)] | 0.86[a)] | 0.65[a)] | 13.01[b)] | 23.74[c)] |
| 8. | *Schima wallichii* | 4.42[1)] | 3.85[1)] | 5.49[b)] | 7.88[b)] | 0.06[a)] | 0.09[a)] | 14.9[c)] | 9.89[b)] | 1.29[a)] | 2.47[a)] | 20.52[b)] | 54.44[d)] |
| 9. | *Macaranga motleyana* | 4.55[2)] | 3.73[1)] | 7.31[b)] | 7.29[b)] | 0.06[a)] | 0.07[a)] | 13.7[c)] | 14.04[c)] | 2.15[a)] | 4.41[a)] | 17.49[b)] | 47.33[d)] |
| 10. | *Shorea balangeran* | 4.52[2)] | 3.69[1)] | 4.84[b)] | 6.32[b)] | 0.07[a)] | 0.07[a)] | 9.5[b)] | 10.12[b)] | 0.86[a)] | 2.47[a)] | 32.38[c)] | 41.19[d)] |

Remarks: [1)] Very acid; [2)] Acid; [a)] Very Low; [b)] Low; [c)] Medium; [d)] High; [e)] Very High (Based on Hardjowigeno, 1995)

stage of the rehabilitation process, which can be shown in the consistent trend of diameter and height growth (Figure 5 and 6) during the four years. *V. pinnata* was one of the dominant plant species in the regeneration process of the tropical rainforest after fire disturbances, which therefore was categorized as a pioneer species (Yassir, van der Kamp, & Buurman, 2010).

From their study, it can be indicated that *V. pinnata* requires a significant amount of sunlight to grow, which is abundantly available at the post-coal mining land at the beginning of the planting process. There was the limited study about the growth of *V. pinnata*, but a study showed that this species is one of the most adaptive plant species growing in the post-tin mining areas in Bangka, Indonesia (Nurtjahya, Franklin, Umroh, & Agustina, 2017). However, if compared to other fast-growing pioneer species such as *Paraserianthes falcataria*, *V. pinnata* has significantly lower height and diameter growth. *P. falcataria*, which is an exotic species, can reach 3.4–16.7 cm of diameter and 3.9–19.6 m of height at the age of about four years in West Java, Indonesia (Krisnawati, Varis, Kallio, & Kanninen, 2011). Nevertheless, the use of fast-growing exotic species in the rehabilitation of post-coal mining area is allowed based on regulations in Indonesia. However, it might have some consequences for biodiversity conservation (Nugroho & Yassir, 2017).

In this study, *F. variegata* and *B. glauca* had poor growth performance, which can be indicated by low survival rates and slowest diameter and height growth among the other species. *F. variegata*, has been used in some tropical forest restoration projects because of its role as a keystone species (Kuaraksa & Elliott, 2013) and pioneer (Kuaraksa, Elliott, & Hossaert-Mckey, 2012). Still, this species seemed unable to adapt in the post-coal mining environment. For example, at the age of 2 years, the growth of *F. variegata* was 6–8 cm diameter, and 6.9 m height in Java applied in the intercropping system (Effendi, 2012). In another study, this species can reach 6.2 cm diameters and 5.1 m height in East Kalimantan in a monoculture planting system (Effendi & Mindawati, 2015). However, the levels of soil degradation in the intercropping and monoculture systems were different than the soil degradation in the post-coal mining areas. The soil in the post-mining area has experienced the heaviest soil degradation compared to the other methods because of heavy machinery activities during surface removal. As a result, the environmental stress in the post-coal mining areas is more intense than ecological stress in the intercropping and monoculture systems, resulting in the slow growth of *F. variegata*.

The inability to adapt in the post-mining areas might also be the case for the poor growth performance of *B. glauca*. Other study showed that *B. glauca*, along with *V. pinnata*, was one of the dominant plant species identified in a secondary forest after ten years regeneration from imperata grassland because of fire disturbances, indicating that *B. glauca* is a pioneer species, demanding direct sunlight for supporting its growth which is abundantly available in the post-mining areas (Komara, Murtinah, & Arbain, 2018; Yassir, 2016). Meanwhile, this species was found in a succession area of degraded forest (Gunawan, 2015). However, *B. glauca* might not be able to grow naturally in the heavily degraded soil such as in the post-coal mining areas. Nevertheless, the information about the growth of *B. glauca* is limited; therefore, it is difficult to compare its growth. A study reported that *B. glauca* could grow to a height of up to 10 m, initially distributed in Okinawa, Japan, Taiwan, Southern part of China, Indochina and the Philippines (Ngueyem, Brusotti, Caccialanza, & Finzi, 2009).

Meanwhile *S. polyanthum* had the most massive diameter growth but moderate height growth compared to the other nine plant species. However, *S. polyanthum* growth in this study was remarkable compared to other studies. A study reported the diameter growth of *S. polyanthum* planted in the restoration of degraded forest in Singapore can reach about 1 cm per year with the average diameter of about 3.5 cm at the age of 4 years (Shono, Davies, & Chua, 2007). The other study showed that at the period of 14

months, *S. polyanthum* had 29.06 cm of height and 0.7 cm of diameter, which was planted in a nature tourism park degraded area with the intercropping system (Sumarhani, 2015). Thus, by considering the level of degradation, which is heavier in the post-coal mining areas than the level of degradation in the other studies, *S. polyanthum* might be able to adapt to the post-mining regions in this study. This species is also known as a medicinal plant (Har & Intan, 2012; Widyawati et al., 2015).

The other species in this study, *Sh. balangeran*, *S. lineatum* and *S. scortechinii* showed consistent moderate diameter and height growth compared to the other species. *S. balangeran* showed a significant increase in diameter and height growth which occurred since the month 42$^{nd}$, after a slow growth during the first 12 months. This indicates that this species might grow well in the post-mining environment. However, the development of *S. balangeran* in the first year in this study is slower compared to several studies. A similar study reported that *S. balangeran* could grow by 19 cm in height and 0.5 mm in diameter during the first year, grown under the shaded area of 4 years old fast-growing plants, Samanea saman (Susilo, 2016), and another study show it grows by 146–149 cm of height and 1.5–3.0 cm of diameter at five years old in post-coal mining land (Lestari, Fiqa, Fauziah, & Budiharta, 2019). This indicates that shaded areas are essential for *S. balangeran* during the first years after planting in the post-mining environment.

On the contrary, another study reported that *S. balangeran* could grow well in the un-shaded imperata grassland, growing by 64–71 cm of height and 8–10 mm in diameter at the first year at various land preparations (Yassir & Mitikauji, 2007). It is important to note that the level of degradation in the imperata grassland was much lower than the level of degradation in the post mining areas, causing significant growth of *S. balangeran* in the first year. In addition, this species is also reported as one of the plants for peat swamp restoration project (Graham, Turjaman, & Page, 2013). Furthermore, *S. balangeran* was said to have about 2.7 cm of stem diameter at the age of 40 months, planted in the peat swamp forest in Central Kalimantan (Turjaman et al., 2011), slightly lower than the diameter growth in this research. This indicates that *S. balangeran* can grow in various environments.

Compared to the other studies, the diameter and height growth of *S. lineatum* in this study was acceptable. A study reported that this species could grow by 0.28 cm in height and 0.76 cm in diameter in the first year (Rahman et al., 2011). Meanwhile, in this study, the height and diameter growth of *S. lineatum* was stagnated during the first year but started to grow consistently from the 2$^{nd}$ to 4$^{th}$ year. This indicates that this species can adapt and grow in a heavily degraded environment such as in the post-mining areas.

Furthermore, *S. lineatum* can also grow in the succession areas in an abandoned grazing site in West Java, Indonesia (Rosleine & Suzuki, 2012). This indicates that this species is pioneer species-meanwhile, works of literature about *Sy. Scortechinii* is limited; therefore, it is hard to compare its growth in another environment.

The other species; *S. wallichii*, *M. motleyana* and *C. myrianthus* had low to the average but the consistent diameter and height growth in the four years compared to the other species. However, it is difficult to evaluate the growth performance of these three species due to limited works of literature. Based on available pieces of literature, *S. wallichii*, *M. motleyana* and *C. myrianthus* can be categorized as pioneer species, appearing after disturbances in a forest ecosystem. For example, *S. wallichii* was reported appearing in an abandoned coal mining site in India, in which the coal extraction was conducted using 'rat-hole' mining method (Sarma, Kushwaha, & Singh, 2010). The other study showed that *S. wallichii* grows in a natural succession area after the volcanic eruption, although this species was not a dominant plant (Suryanto, Zaki, Azani, & Azmy, 2010).

Meanwhile, another study shows that *C. myrianthus* was a plant species in the secondary forests (Yusuf & Purwaningsih, 2012). Also, *M. motleyana* was a pioneer species that reported to dominantly inhabit the low to intermediate

disturbance (Slik, Keßler, & van Welzen, 2015) level forest. Nevertheless, in the high level of disturbances such as mining activities in this study, in which the soil experience compaction, these tree species might still be able to grow, but they need time to adapt to the post-mining environment during the first year.

Increasing plant growth needs input technology. Soil management that preserves soil nutrients and prevents acidification is likely key to the success of reforesting post-mining land (Woodbury et al., 2020). The use of mycorrhizae can be a solution to improve soil quality and plant growth. Application of mycorrhizae could improve some chemical soil properties such as pH, soil organic C, total soil N, and available P (Agus, Primananda, Faridah, Wulandari, & Lestari, 2019; Wulandari, Saridi, Cheng, & Tawaraya, 2016).

## IV. CONCLUSION

In conclusion, almost all plant species in this study have been able to grow and to adapt to the post-coal mining land, and only 2 out of 10 species were unable to adapt in this environment. In this study, *V. pinnata* had the most significant growth performance compared to the other nine species. *S. polyanthum*, *S. scortechinii* and *S. lineatum* also had a good growth performance in the post-coal mining land. Even though as a climax forest species, *S. balangeran* had an excellent performance in an open area such as post-coal mining land.

In the early period after plantation, all species experienced slow growth due to adaptation to the post-mining environmental condition, after 12 months the development of the ten species increased at a variable rate. Only two species; *B. glauca* and *F. variegata* experienced a decrease in diameter and height growth two years after planted.

*V. pinnata*, *S. polyanthum*, *S. scortechinii*, *S. lineatum,* and *S. balangeran* can be recommended as revegetation plants for the rehabilitation of post-coal mining land. Trials should be undertaken on other local trees and local climax trees to support the success of the rehabilitation of post-coal mining land mainly located in the forest area.

## ACKNOWLEDGEMENT

The authors would like to thank the leaders and employees of PT Singlurus Pratama, which helped the field implementation of this study. Also thanks to Yusuf Wibisono, Mujianto, Ermansyah, Deny Adi Putra, Suhardi, Agung Siswanto and Iman Suharja who helped in collecting data in the field. The authors are also very grateful to the Chairman of Balitek KSDA and staff who helped in the administration process of the study. This study was funded by DIPA Balitek KSDA during 2012-2016 financial years.

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# REMOTE SENSING FOR URBAN TREE CANOPY CHANGE DETECTION WITH LANDSAT SATELLITE DATA IN NNAMDI AZIKIWE UNIVERSITY AWKA – NIGERIA

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Received: 26 April 2020, Revised: 19 October 2020, Accepted: 21 October 2020

REMOTE SENSING FOR URBAN TREE CANOPY CHANGE DETECTION WITH LANDSAT SATELLITE DATA IN NNAMDI AZIKIWE UNIVERSITY, AWKA – NIGERIA. Urban tree canopy within a university boundary is a measure of the university's tree cover as a percentage of its total land area. The overall objective of the present study is to conduct a spatio-temporal change analysis of urban tree canopy in Nnamdi Azikiwe University Awka-Nigeria. Landsat data of years 1991, 2001, 2011 and 2019 were analysed using Maximum Likelihood Classifier and Confusion Matrix Spatial Analyst in ArcGIS 10.7.1 software. In terms of tree cover loss, there is a steady rate of decrease from -31.59 ha between 1991 and 2001; -82.32 ha (2001/2011) and -64.53 ha (2011/2019). Whereas, at an initial land area of 9.40 ha in 1991, physical infrastructural development is progressively increased with 16.92 ha between 1991 and 2001; 43.79 ha 2001/2011 and 12.37 ha between 2011 and 2019. The dominant drivers of tree cover change in the study area related to the expansion of physical infrastructures and sprawling agriculture as a result of encroachers into the study area. In conclusion, tropical forests within university campuses face many threats, such as those posed by unregulated physical infrastructural development and a lack of investment and management of forest relics. As a recommendation, Nigerian universities should invest and conserve their existing forested landscapes towards promoting land resources in line with Sustainable Development Goals number 15 (SDG-15) strategies.

Keywords: Change Detection, Kappa coefficient, Landsat Remote Sensing, Nnamdi Azikiwe University Awka, Tree Canopy, Urban sprawl

*PENGINDERAAN JAUH UNTUK MENDETEKSI PERUBAHAN KANOPI POHON KAWASAN PERKOTAAN MENGGUNAKAN DATA SATELIT LANDSAT DI NNAMDI AZIKIWE UNIVERSITY AWKA – NIGERIA. Kanopi pohon di kawasan perkotaan seperti kanopi pohon di perbatasan wilayah suatu universitas merupakan ukuran tutupan pohon wilayah universitas sebagai suatu persentase dari total luas lahan. Penelitian ini menganalisis perubahan spatio-temporal dari kanopi pohon perkotaan di wilayah Nnamdi Azikiwe University Awka-Nigeria. Data Landsat tahun 1991, 2001, 2011, dan 2019 dianalisis menggunakan Maximum Likelihood Classifier dan Confusion Matrix Spatial Analyst pada perangkat lunak ArcGIS 10.7.1. Dalam hal penurunan tutupan pohon, hasilnya menunjukkan penurunan yang stabil dari -31,59 ha antara tahun 1991 dan 2001; -82,32 ha (2001/2011) dan -64,53 ha (2011/2019). Pada tahun 1991, luasan awal seluas 9,4 ha pada tahun 1991, pembangunan infrastruktur fisik semakin meningkat seluas 16,92 ha antara tahun 1991 dan 2001; 43,79 ha 2001/2011 dan 12,37 ha antara tahun 2011 dan 2019. Pendorong utama perubahan tutupan pohon di wilayah studi terkait dengan perluasan infrastruktur fisik dan perladangan berpindah dari penduduk desa yang bertetangga, melakukan perambahan ke wilayah yang dianalisis. Dari penelitian dapat disimpulkan bahwa hutan tropis di kawasan kampus termasuk yang menghadapi banyak ancaman, seperti yang ditimbulkan oleh pembangunan infrastruktur fisik yang tidak diatur dan kurangnya investasi dan pengelolaan peninggalan hutan. Untuk itu, disarankan agar universitas-universitas di Nigeria berinvestasi dan melestarikan lanskap hutan yang ada untuk mempromosikan pengelolaan sumber daya lahan, mengikuti pencapaian strategi tujuan pembangunan berkelanjutan nomor 15 (SDG-15).*

*Kata kunci: Deteksi perubahan, koefisien Kappa, penginderaan jauh Landsat, Nnamdi Azikiwe University Awka, kanopi pohon, perluasan kota*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.99-112 

## I. INTRODUCTION

The growing human populations require sustainable space and natural resources management. Natural resources management (NRM) is the sustainable use of resources that come from nature (i.e. forest, agriculture, land, water and air) towards optimising their economic, social and cultural values for both present and future generations. Thus, the principle of NRM further conforms to the concept of sustainable development which requires that policies are executed towards achieving economically viable, socially acceptable and ecologically sound natural resources management.

Specifically, the Sustainable Development Goals (SDGs) number 15, (on landcover/ land-use change and terrestrial biodiversity conservation), is pivotal for natural resources management actions taken at both corporate and public spheres of the human society (World Bank, 2009). For instance, the increasing demands for natural resources by humans are primarily supplied in un-sustainable manners, especially in developing countries. As such, a myriad of social, economic and environmental consequences arising from competing claims for natural resources exploration, usage and consumption (World Bank, 2009). For example, the effects of natural events combined with the ever-increasing human requirements for economic activities and urban sprawl, are putting high demands on environmental resources. According to Livesley, Escobedo and Morgenroth (2016), tropical forest availability has significantly dwindled through urban sprawling.

Any university campus could be an urban prototype, because, of the degree of urban indices that are operational within its boundary. FAO - Food and Agriculture Organization of the United Nations (2012) and Zubair (2006) both assert that urban sprawl is a crucial driver of tropical deforestation in the world. According to Ogbodo, Tembe and Peter (2017) expansion of university-based infrastructures is among the competing factors that drive tropical deforestation in the southern part of Nigeria. This situation creates conflicts that result in unsustainable consumptions of land resources, thereby, placing high pressures, particularly on the availability of tropical forest (World Bank, 2009; Zubair, 2006). Furthermore, tropical deforestation is an issue of major concern that has numerous consequences on thermal comfort (Xiao et al., 2006; Xiaojun, 2011). Severe heat has negative impacts on human well-being and efficiency; especially, as it relates to teaching and learning within a university campus. Naturally, the sunlight that produces heat waves is captured by the forests.

A forest is any land area of more than 0.5 hectares with trees higher than five meters and a canopy cover of more than 10% (FAO, 2012). Despite that, tropical forests have limited spatial extent; they continue to play critical roles in global exchanges of energy, in biogeochemical cycling (Ogbodo, Oke & Dagba, 2015). Literature asserts that forests are the largest terrestrial sequester of carbon dioxide ($CO_2$) from the atmosphere (Ontl et al., 2020). However, it has become increasingly alarming that impacts of anthropogenic climate change are compromising the supplies of ecosystem services from dwindling forest resources; thus threatening human societies in many sectors coupled with their attendant effects on human survival and well-being. FAO (2012) estimated that around thirteen (13) million hectares per year had been lost to deforestation activities in the Afrotropic[1] region.

FAO (2012) defined the term deforestation as the conversion of forest cover into the other land use. In other words, it is a process by which forest cover is converted into another land cover type such as bare land, farmland, and building. Thus, forest cover conversion is of great concern to scientists because, among other environmental issues, it often contributes to increased levels of global warming (short term) and climate change (over two and a half decades) (Ogbodo, Wasige, Shuaibu, Dube & Anarah, 2019; Wimrly and Ohmann, 2004). The environmental issue arises because when forests are cleared or degraded, their stored

1 = Tropical area

carbon is released into the atmosphere as carbon dioxide ($CO_2$) (FAO, 2001). In other words, $CO_2$, a greenhouse gas that contributes to global warming, is captured by forest trees.

Forest is, therefore, a type of landcover at any given area. FAO (1997) defined, landcover as (a) vegetation (example: natural or planted vegetation); (b) human-made constructions (such as buildings); and (c) water bodies, rocks, icecaps, bare land and dunes; that are found on the earth surface. Studying land cover change is technically referred to as land cover change detection. According to Kotoky, Dutta & Borah (2012), a land cover change is identified by observing the state of an Earth's features at different time intervals. Usually, there are five causes of land cover change (Lambin & Geist, 2006): (a) ecological and geomorphological processes such as vegetation succession and soil erosion; (b) human-induced alterations of vegetation cover and landscapes such as deforestation and forest degradation; (c) long-term natural changes in climatic conditions; (d) inter-annual climate variability; lastly (e) greenhouse effects resulting from human-induced activities. Signs of land cover change can be observed physically, with Earth Observation (EO) technology (Sonti, 2015).

Earth Observation (EO) technology such as satellite remote sensors, in conjunction with ground-based data, are capable of observing, monitoring, measuring and modelling various components of natural ecosystems. For instance, remote sensing methods are widely used to acquire data about the earth surface from a distance either from a satellite or airborne platform (Ogbodo et al., 2019). According to Lillesand, Kiefer, and Chipman (2008), several remotely sensed data used for mapping and spatial analysis are collected as reflected electromagnetic radiation. They are afterwards, processed into a digital image that can be overlaid with other spatial data. To assess and monitor human-induced land cover changes, one must in effect separate the signal of inter-annual land cover change from the regular seasonal changes that one would expect to observe in the tropical forest (Sonti, 2015). Change detection methods with remote sensing are basically grouped into visual analysis and classification approaches, among a few others (Lambin & Geist, 2006).

The effects of natural events combined with the ever-increasing human requirements for economic activities and urban sprawl are putting high demands on tropical forests. This situation creates conflicts that result in over-depletion of forest resources. The impacts of anthropogenic climate change are compromising with the supplies of ecosystem services from dwindling natural resources; thus threatening human societies in many developing countries coupled with their attendant effects on human survival and well-being.

Nevertheless, there is an absence of requisite information on forest cover change rates for Nigerian universities. Scarce state-of-the-art information on forest cover dynamics in any Nigerian university system can limit effective monitoring of their forest resource; investment opportunities for contemporary forestry research and conservation; and forestry policy-marking to support the Reducing Emissions from Deforestation and forest Degradation (REDD+) global climate change framework.

The overall objective of this study is to assess the Spatio-temporal change analysis of urban tree canopy in Nnamdi Azikiwe University Awka-Nigeria with Landsat satellite remote sensing data, 1991–2019. The outcomes of this study could be useful for the promotion of climate-smart campuses as a panacea for a university-based strategy for achieving sustainable climate change mitigation and adaptation in Nigeria.

## II. MATERIAL AND METHOD

### A. Study Area

1. Description of the Study Area

Nnamdi Azikiwe University Awka (NAU) (Figure 1) is a Federal University established in 1991 and located in the south-eastern part of Nigeria, precisely, within a suburban environment in the Anambra State's Capital City of Awka. The spatial size of the NAU

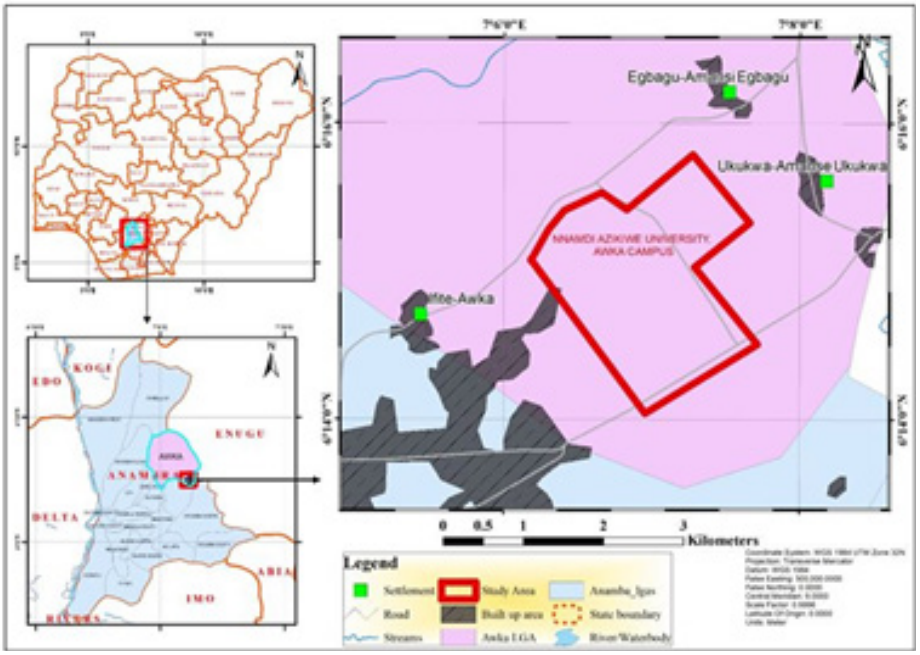


Figure 1. Map of study area showing Nnamdi Azikiwe University Awka-Nigeria

Awka campus is approximately 4.99 km$^2$ (an equivalent of 499 Hectares)[2]. The study area is situated on the geographic coordinates of 6°14'38.4"N and 7°07'18.7"E.

2. Vegetation of Awka

Awka, the seat of NAU, is in the tropical rainforest zone of Nigeria. Awka is situated on a fertile tropical valley. The original rainforest cover has been lost due to expansion of farming systems and human settlements. Thus, derived savannah grassland currently dominates the original primary tropical rainforest estates.

3. Climate of Awka

The study area experiences two distinct climatic seasons brought about by the two predominant winds that rule Awka, namely: the south-western monsoon winds from the Atlantic Ocean and the north-eastern dry winds from across the Sahara desert. The monsoon winds from the Atlantic create six months of heavy tropical rains, which occur between April and July, followed by a short dry period in August lasting one to two weeks (nowadays) with the rains resuming in September and ceasing in October. The wet (rainy) season is followed by five months of the dry period from November to March marked by the Harmattan wind. Harmattan is a dry, and dusty wind and a grey haze characterise it. The haze usually limits visibility and blocking the sun's rays. Generally, the intensity of Harmattan in the study area increases from the beginning of every November until January.

4. Temperature

Extreme dry heat period occurs around February and March. The temperature in Awka is generally 27–30$^o$C between May and January but rises to 32–34$^o$C between February and April, with the last few months of the dry season marked by intense heat.

## B. Remote Sensing Data

Four Landsat imagery, namely: Landsat 4 Thematic Mapper (TM), dated January 7, 1991; Landsat 7 Enhanced Thematic Mapper (ETM+) of February 19, 2001; ETM+

[2] It is worth-mentioning that, at the time of writing this paper, there is no previous literature on the internet which has provided an actual size of the study area. Thus, we computed the size of the study area (in Sq.km and hectares) using the Geographical Information System (GIS) Shapefile (i.e. the red polygon in Figure 1). The above referenced shapefile was obtained from the NAU's Department of Geoinformatics and Surveying.

(dated, January 30, 2011) and Landsat 8 Operational Land Imagery (OLI) of January 12, 2019; were acquired from the United States Geological Survey (USGS) Archive which is accessible at http://earthexplorer.usgs.gov. The selected image dates were based on image-data availability over the study area and low percentage-cloudiness on the images. The afore-listed satellite images were analysed for forest cover change phenomenon (Akbari & Mamanpoush, 2006; Yuan, Sawaya, Loeffelholz & Bauer, 2005). The spatial resolution of one pixel of the images used is 30 m.

### C. Geometric Correction

The provider already did the geometric correction for the Landsat images before their acquisition for this present study. The used remotely sensed data were geometrically corrected to the World Geodetic System 1984 (i.e. WGS-84) datum. In the present study, satellite images were re-projected to WGS84-UTM Zone32N to ensure accurate metric measurements of the landcover classes in the study area.

### D. Collection of Ground-Truthing Data for Validation

To validate the classified maps, ground truth (reference) data together with their Global Positioning System (GPS) coordinates were obtained from Google Earth (Tilahun & Teferie, 2015). Google Earth was used to ascertain how many ground truth pixels are correctly classified. Ground truthing was done in comparison with the visual analysis of the Landsat imagery used and based on the authors' prior knowledge of the area. Therefore, fifty (50) regions of interest (AOI) were purposefully sampled per land cover class in the study area (Lillesand et al., 2008).

### E. Remote Sensing for Forest Cover Classification and Change Detection

1. Satellite Image Classification

The unsupervised classification was first performed on the images. The core objective of unsupervised image classification is to automatically categorise all pixels in an image into land cover classes. In the present study, the Landsat classification legend comprises of five land cover classes, namely built-up area, forest land, agricultural land, bare land and grassland in the study area. The afore-mentioned landcover classes were made based on spectral characteristics of the Landsat image analysis (Akbari & Mamanpoush, 2006). Next, supervised classification was done by following three stages that included training data sets, classification and output. Training samples were taken for each land cover type that was identified on the supervised classification output. The classification was done by using maximum likelihood classifier (Torahi, 2013; Congalton, Oderwald, & Mead, 1983).

2. Change Detection Analysis:

In order to detect, assess, and map the forest cover change in the study area, from the period from 1991 (year of Nnamdi Azikiwe University establishment) to 2019 (current year of this research), various datasets from Landsat sensors of TM, ETM, and OLI were used. Change detection is the process of identifying differences in the state of an object or phenomena by observing it at different times (Yuan et al., 2005; Singh, 1989). From visual and digital interpretations of the satellite images, different land cover categories were distinguished in the study area, and emphasis was mainly given to green and infrastructure classes. Hence, forest areas and urban sprawl indices such as buildings and roads were detected and analysed from the imagery, based on Lung and Schaab (2010) land cover classification techniques. The percentage landc over change (trend) was computed with Equation 1 (Zubair, 2006):

$$\%\ Landcover = \frac{Observed\ landcover\ change}{Sum\ of\ landcover\ change} x\ 100 \quad ...(1)$$

### F. Computation of Accuracy Assessment

The confusion matrix is usually used as the quantitative method of characterising satellite image classification accuracy (Adam, Elhag & Salih, 2013). A confusion matrix is a table that shows the correspondence between the

classification results and the referenced image-data (Ukrainski, 2016). Diagonal cells of a confusion matrix table contain the number of correctly identified pixels. Therefore, Equation 2 was applied (Ukrainski, 2016) in determining the overall accuracy in the percentage of the land cover classification.

$$Overall\ Accuracy\ (\%) = \frac{Number\ of\ correctly\ sampled\ pixels\ per\ AOI}{Total\ number\ of\ sampled\ pixels\ of\ the\ AOIs} \quad .(2)$$

Similarly, user's accuracy and producer's accuracy types, including errors of commission and errors of omission were all calculated in the present study. According to Ukrainski (2016), for any class, errors of commission occur when a classification procedure assigns pixels wrongly to any other land cover class. A number of pixels mistakenly assigned to a class can be found in column cells of the class above and below the main diagonal. Furthermore, for any land cover class, errors of omission occur during class overlapping pixels. From the main diagonal, the omitted pixels is located in the row cells to the left and right of the matrix.

### G. Computation of Kappa Coefficient

Ukrainski (2016) explained the Kappa coefficient (denoted as K) as a measure of how the classification results compare to values assigned by chance. K reflects the difference between the actual agreement and the agreement expected by chance. It can take values from 0 – 1. If Kappa coefficient equals to 0, there will be no agreement between the classified image and the reference image. Therefore, the higher the Kappa coefficient, the more accurate the classification is. Equation 3 (Lillesand et al., 2008) was used to determine the Kappa statistic for our classification analyses.

$$Kappa = \frac{Observed\ accuracy - Chance\ agreement}{1 - Chance\ agreement} \quad .(3)$$

## III. RESULT AND DISCUSSION

### A. Results

#### 1. Analysis of Land Cover Trend, from 1991 – 2019, in the Study Area

After classifying the satellite images from four different dates, land cover map for the periods, results of how much areas of land cover types changed in the study area is presented in Table 1. From Table 1, the classification shows the initial land cover types as follows: 56.16% (forest cover), 26.05% (grassland), 14.87% (cropland) 1.88% (built-up area = physical infrastructure) and 1.03% (bare land). Figure 2 shows the landcover map for the period under investigation.

#### 2. Comparing the Forest Cover and Build-up Area Changes in the Study Area

Spatial pattern in Figure 3 indicates a steady tree cover loss from - 31.59 ha (1991/2001) through -82.32 ha (2001/2011) to -64.53 ha (2011/2019), and physical infrastructural development (Figure 4) progressively increased with 16.92 ha between 1991 and 2001; 43.79 ha 2001/2011 and 12.37 ha during 2011 – 2019 period.

Table 1: Landcover change detection 1991 to 2019

| Landcover type | 1991 | | 2001 | | 2011 | | 2019 | | Tree cover Area Difference (Ha) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | [a]Area (Ha) | % | [b] Area (Ha) | % | [c] Area (Ha) | % | [d] Area (Ha) | % | b-a | c-b | d-c |
| Agric. land | 74.19 | 14.87 | 45.36 | 9.11 | 53.19 | 10.66 | 59.00 | 11.82 | -28.83 | 7.83 | 5.81 |
| Bare land | 5.16 | 1.03 | 20.21 | 4.06 | 31.58 | 6.33 | 108.67 | 21.77 | 15.05 | 11.37 | 77.09 |
| Built up | 9.40 | 1.88 | 26.32 | 5.28 | 70.11 | 14.05 | 82.48 | 16.53 | 16.92 | 43.79 | 12.37 |
| Forest | 280.29 | 56.16 | 248.70 | 49.93 | 166.38 | 33.34 | 101.85 | 20.41 | -31.59 | -82.32 | -64.53 |
| Grassland | 130.01 | 26.05 | 157.54 | 31.63 | 177.85 | 35.63 | 147.09 | 29.47 | 27.53 | 20.31 | -30.76 |
| Total | 499.06 | 100.00 | 498.13 | 100.00 | 499.09 | 100.00 | 499.09 | 100.00 | | - | |

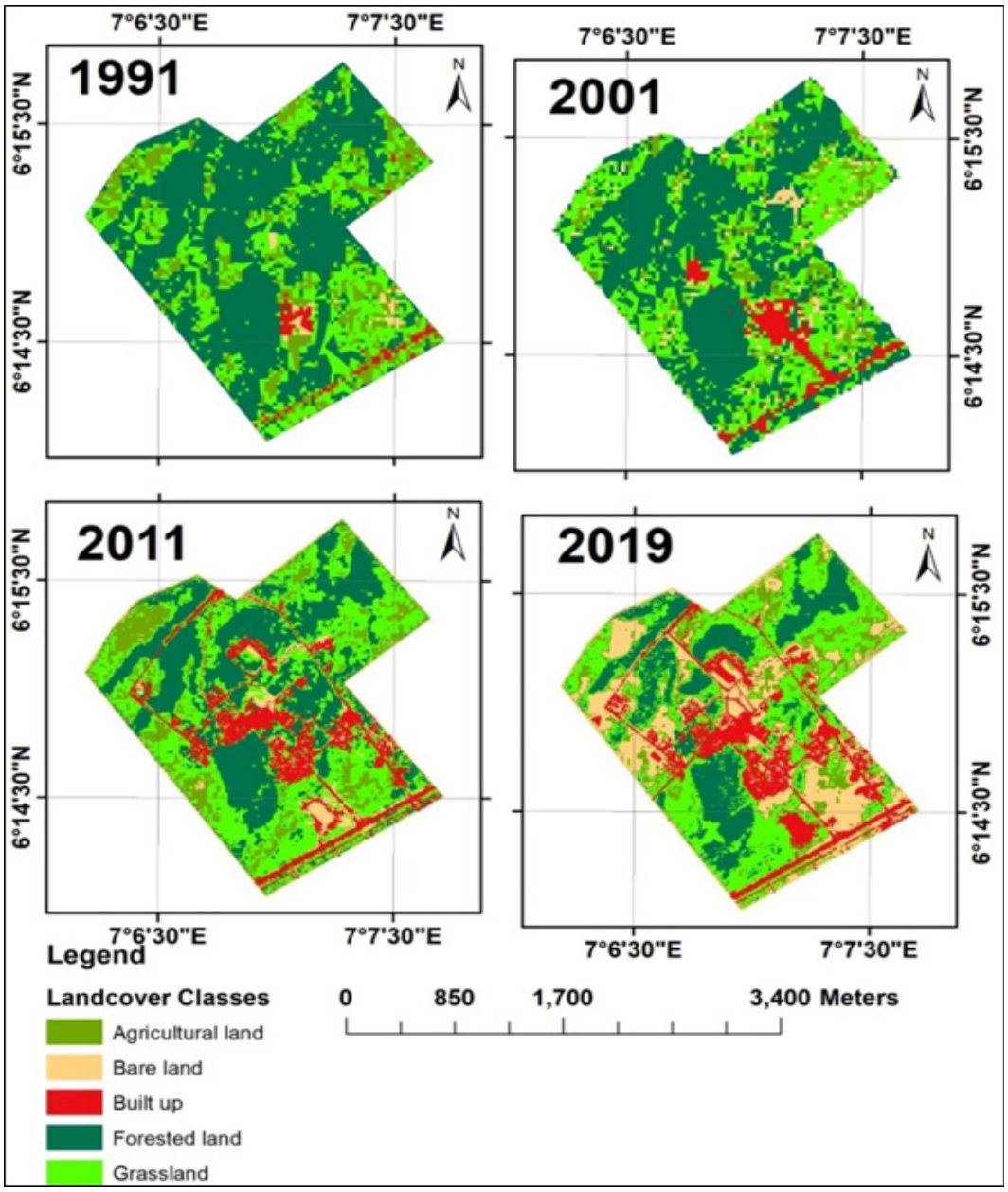


Figure 2. Landcover classification map of the study area

## C. Results of the Computation of Accuracy Assessment

Figure 5 presents the distribution and the referenced data for 2019 used for validation of the processed remotely sensed data in this present study. Table 2 presents the results of accuracy assessment of the Landsat 8 and the referenced data from Google Earth coupled with visual ground observations in 2019 (i.e. the year of ground-truthing data).

## D. Result of Kappa Coefficient Computation

Therefore, the Kappa coefficient value means that there is 98% classification agreement between the classified Landsat 8 imagery and the referenced data for each of the years 1991 and 2019. In summary, the Kappa statistical results for the 1991 and 2019 Change Detection analysis (Figure 6) stands at 0.981 and 0.982, respectively (Table 3); meaning that both the

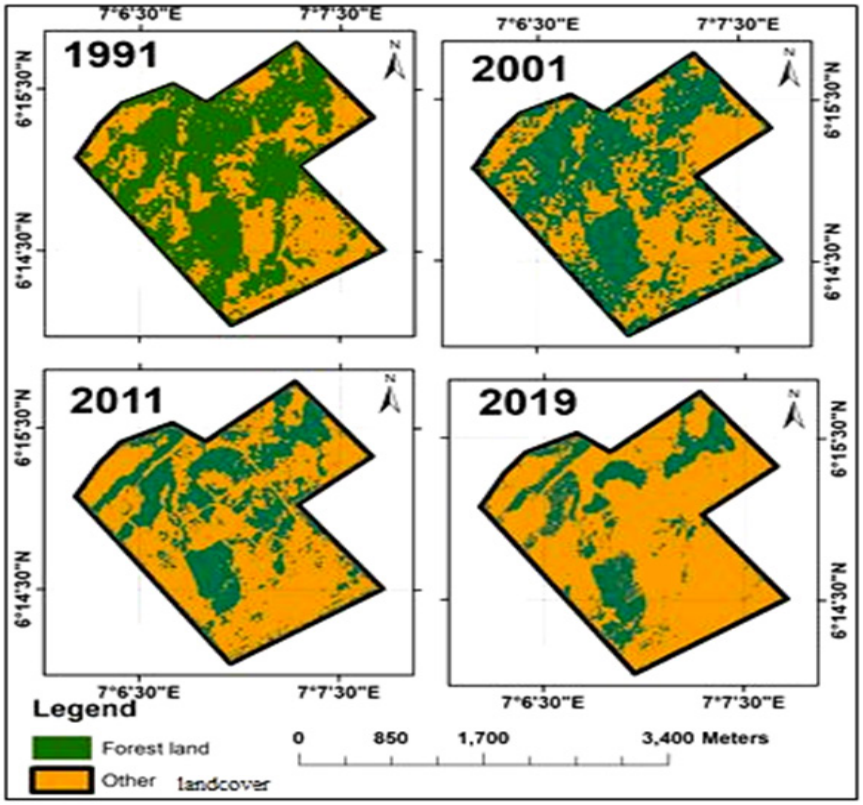


Figure 3. Forest cover map of the study area

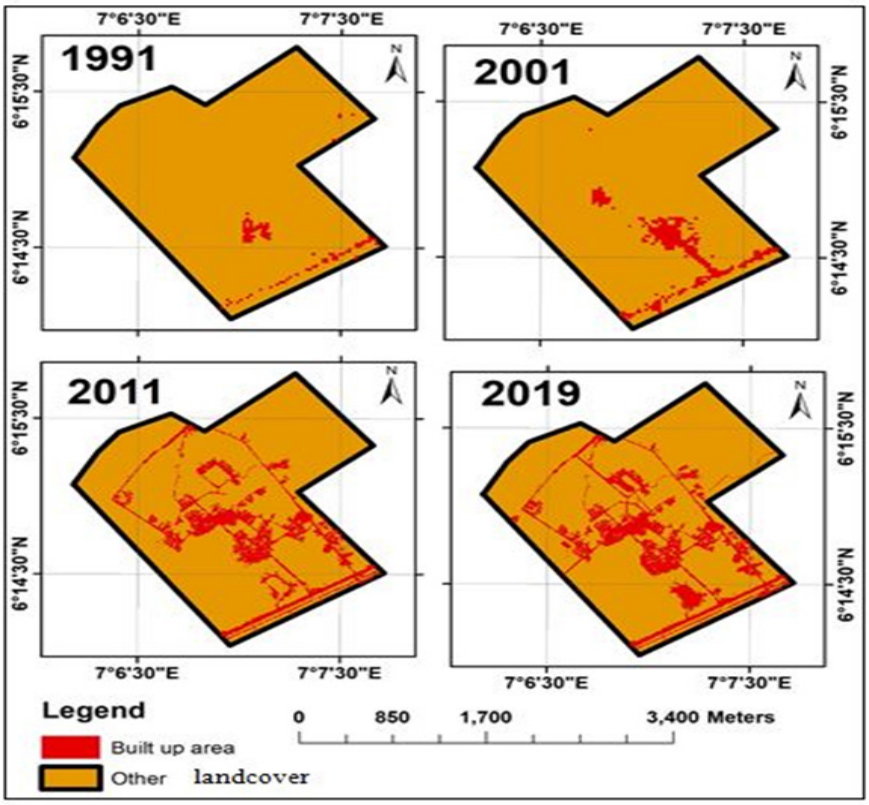


Figure 4. Built-up area change map

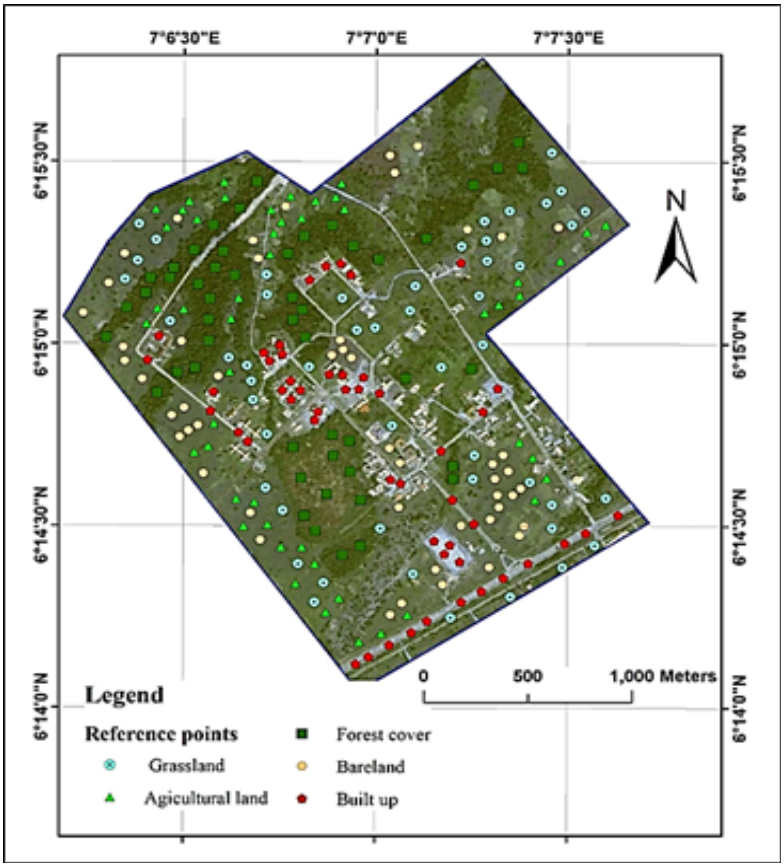


Figure 5. Distribution of ground control points in the study

Table 2: Confusion matrix of classification analysis for the Landsat image 2019

| | | Ground truthing data | | | | | | Omission Error | Users Accuracy (%) |
|---|---|---|---|---|---|---|---|---|---|
| Classified Landsat image, 2019 | Class | Forest | Agric. land | Grassland | Built-up | Bare land | Total | | |
| | Forest | 44 | 4 | 2 | 0 | 0 | 50 | 0.120 | 88 |
| | Agric. land | 0 | 38 | 1 | 1 | 0 | 40 | 0.050 | 95 |
| | Grassland | 7 | 13 | 30 | 0 | 0 | 50 | 0.400 | 60 |
| | Built-up | 0 | 1 | 0 | 34 | 9 | 44 | 0.227 | 77 |
| | Bare land | 0 | 2 | 8 | 0 | 29 | 39 | 0.256 | 74 |
| | Total | 51 | 58 | 41 | 35 | 38 | 223 | Overall Accuracy = 78.50% | |

$$Kappa = \frac{(44+38+30+34+29) - [(51*50)+(58*40)+(41*50)+(35*44)+(38*39)]}{1 - [(51*50)+(58*40)+(41*50)+(35*44)+(38*39)]}$$

$$= \frac{(175) - (9942)}{1 - (9942)}$$

$$= \frac{-(9767)}{-(9941)}$$

$$\because \boldsymbol{Kappa = 0.982}$$

Table 3: Confusion matrix of classification analysis for the Landsat image 1991

| Classified Landsat image, 1991 | Ground truthing data | | | | | | Omission Error | Users Accuracy (%) |
|---|---|---|---|---|---|---|---|---|
| | Class | Forest | Farmland | Grassland | Built-up area | Bare land | Total | |
| | Forest | 46 | 3 | 0 | 2 | 0 | 51 | 0.100 | 90 |
| | Farmland | 2 | 43 | 4 | 0 | 0 | 49 | 0.122 | 88 |
| | Grassland | 1 | 2 | 45 | 0 | 0 | 48 | 0.063 | 94 |
| | Built-up area | 0 | 0 | 0 | 48 | 0 | 48 | 0.000 | 100 |
| | Bare land | 0 | 0 | 0 | 1 | 49 | 50 | 0.020 | 98 |
| | Total | 49 | 48 | 49 | 51 | 49 | 246 | Overall Accuracy = 93.90% | |
| Commission Error | | 0.061 | 0.104 | 0.082 | 0.059 | 0 | | | |
| Producer's Accuracy (%) | | 94 | 90 | 92 | 94 | 100 | | Kappa: 0.981 | |

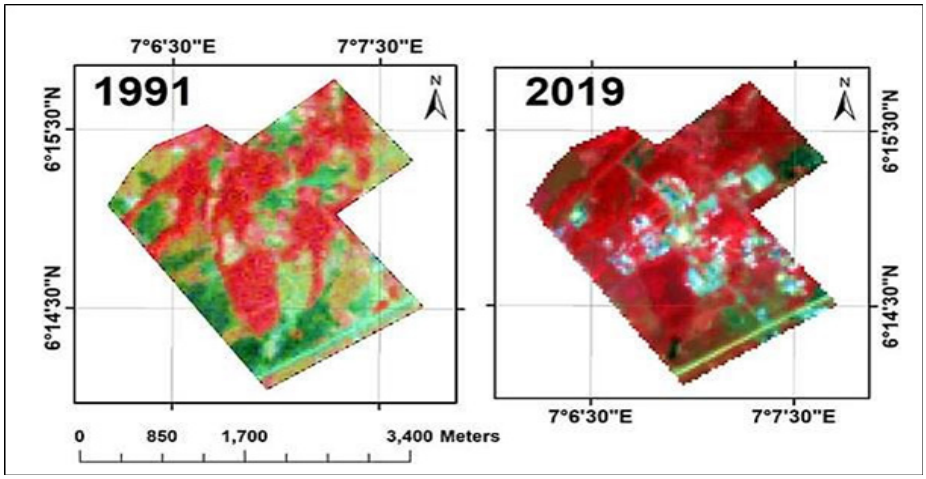


Figure 6. Overview of pronounced buildings in 2019 as against 1991 Landsat image

classified imagery and the reference datasets are very close, expressing high accuracy.

In conclusion, there is a tremendous rise in human-made structures in 2019 as compared to 1991 (Figure 7) in the case study area. The change in the built-up area includes administrative buildings, lecture halls, medical facility, worship buildings, road infrastructure, student hotels, workshops and other buildings such as university auditorium, etc. The university authority carried out the overall landcover change within the period investigated without a corresponding investment rate of reforestation. Thus, tropical forests within the Nigerian university campus face many threats, such as those posed by unregulated physical infrastructural development and a lack of investment and management of forest relics.

## B. Discussion

According to Nigeria's Federal Department of Forestry (2019), Nigeria's forest vegetation declines due to deliberate removal of trees to pave the way for mineral exploitation and sprawling agriculture. Furthermore, Ogbodo et al. (2017) express that expansion of infrastructures is one of the factors that drive deforestation in public universities that are majorly situated within the natural forest part of Southern Nigeria. The finding of this study is in agreement to Ogbodo et al. (2017) which asserts that huge physical development projects

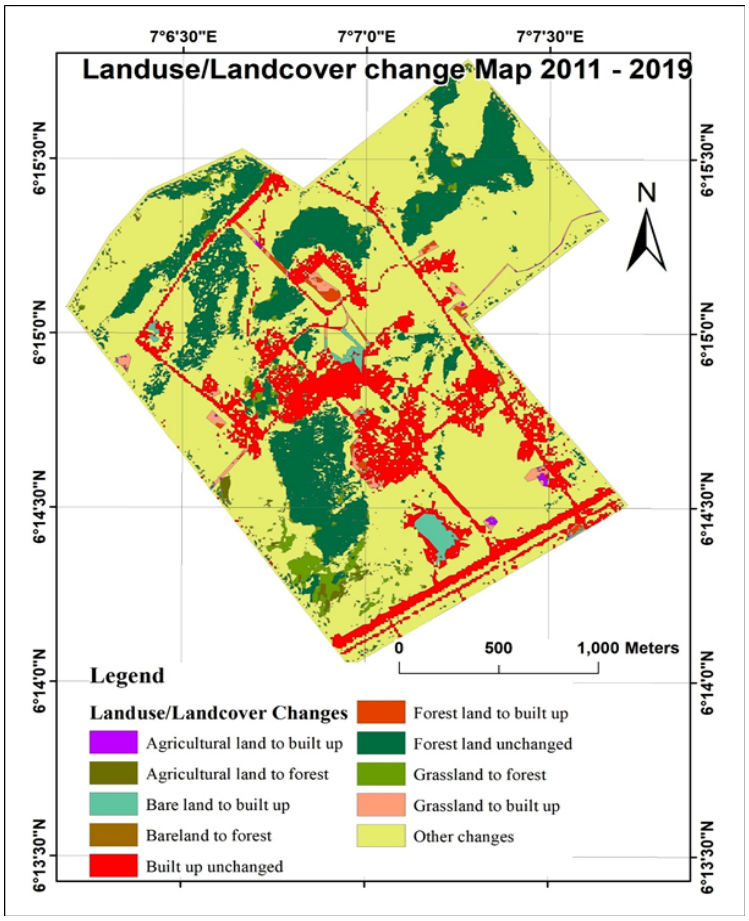


Figure 7. Landcover change map of Nnamdi Azikiwe University Awka Campus

in public universities are primarily financed by Tertiary Education Trust Fund (TETFund) which usually leads to the replacement of forestland with buildings such as lecture theatres, administrative blocks, student hotels, without corresponding TETFund-sponsored reforestation or afforestation in Nigerian universities. Furthermore, Zubair (2006) expressed that, massive deforestation rates are experienced within the Afrotropic region of the world due to human-induced activities from sprawling agriculture and increase in built-up areas. Therefore, sustenance of the foregoing scenario within Nigerian universities campuses will negate the principle of sustainable development.

Brundtland (1987) defined sustainable development as a development that meets the needs of the present human generations without jeopardizing future generations from meeting their own needs of the same environmental resources. Thus, imbibing the tenets of sustainable development can be achieved in Nigeria when university authorities mainstream urban forestry guidelines into their master plans on physical infrastructural developments. According to Sustainable Development Solutions Network (SDSN) – Australia (2017) universities should always commit to ensuring safe and green campuses. To achieve SDG-15 in Nigeria, practising of urban tree canopy strategies by university authorities is, therefore, highly imperative. Urban tree canopy is referred to as a measure of a university's tree cover as a percentage of the total land area (Salbitano, Borelli, Conigliaro & Chen, 2016). Thus, mapping of urban tree canopy can support vital thermal heat comforts within university communities across Nigeria. Thermal comfort on campus cities of universities has become more and more important, especially within the context of global warming and rapid urban

sprawling (Xiao et al., 2006). Urban thermal comfort would influence human outdoor activities and the utilisation of urban spaces (Xiaojun, 2011). Severe heat has negative impacts on human well-being and efficiency; especially, as it relates to teaching and learning within a university campus.

Therefore, it is essential to infer in the present study that, an increase in forest coverage within any university campus could be one of the most effective ways of lowering heat waves. Evans (2014) expresses that trees serve as a natural air conditioner. This is because; the cooling effect capability of a single tree can be equivalent to the output capacity of ten (10) room-sized air conditioners when operating 20 hours a day.

Based on the foregoing, in adherence to the slogan of the United Nations Sustainable Development Goals (SDGs) of leaving *no one/ sector* behind, measuring of the ever-changing forest cover pattern within university campuses in tropical regions is imperative for actualising the SDGs, particularly, SDG-11 (sustainable cities/campuses), SDG-13 (combating climate change and its impacts), and SDG-15 (sustainable forest conservation) by 2020 in Nigeria.

## IV. CONCLUSION AND RECOMENDATION

In conclusion, therefore, there is a tremendous rise in human-made structures within the study area without a corresponding investment in reforestation projects. With an initial land area of 9.40 ha in 1991, physical infrastructural development progressively increased with 16.92 ha in the 1991/2001 period; 43.79 ha 2001/2011 and 12.37 ha between 2011 and 2019. The Kappa statistical results for the 1991 and 2019 Change Detection analysis respectively stand at 0.981 and 0.982; meaning that, both the classified imagery and the reference datasets are in full agreement with each other. In conclusion, there is a tremendous rise in human-made structures at the study area within the period investigated, without a corresponding investment rate in reforestation. Thus, tropical forests within the Nigerian university campus face many threats, such as those posed by unregulated physical infrastructural development and a lack of investment and management of forest relics.

For this study, three salient recommendations are at this moment proffered as a way forward. Firstly, there is a need to ascertain the availability and distribution of trees-outside-the-forest within the study area before concluding as to the capability of Nnamdi Azikiwe University Awka-Nigeria to achieve SDGs (numbers 13 and 15) by 2030. Secondly, it is imperative that the Nnamdi Azikiwe University's master-plan is updated to meet-up with global best practices on new forest conservation measures. Thirdly, there is a need to scale-up this study to other universities in Nigeria towards obtaining a holistic outcome for sustainable forestry development goals in Nigeria.

In this research, the 'water' feature was not classified as one of the land cover types in the study area because the cloud-free Landsat imagery available for the study area falls within the dry season period. Future research needs to combine radar and Landsat data towards obtaining accurate land cover classes in the wet-season period in the study area.

## ACKNOWLEDGEMENT

We would like to acknowledge the valuable contributions made by the editors and all the reviewers towards improving this article.

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# STORAGE TECHNIQUES OF CEMPAKA WASIAN (*Magnolia tsiampaca* (Miq.) Dandy) SEEDS

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Received: 2 February 2020, Revised: 22 October 2020, Accepted: 22 October 2020

STORAGE TECHNIQUES OF CEMPAKA WASIAN (*Magnolia tsiampaca* (Miq.) Dandy) SEEDS. Cempaka wasian is a type of woody tree which has a substantial historical value associated with the local culture of the Minahasan people. The improvement of cempaka wasian plantation is essential due to their enormous importance and usage. This study aims to discover the impacts of duration, storage room, and containers used for the appropriate storage of cempaka wasian seeds. The design used in this study is entirely randomized design organized with a factorial pattern which consists of three factors; 1) storage duration, 2) storage room and 3) storage containers. Duration of storage was 2, 3, 4, and 5 months; meanwhile, storage rooms comprised of refrigerators, chambers, and air-conditioned room. Moreover, storing containers consisted of calico cloth, aluminium foils, and plastic bags. The study finding indicates that the seeds of cempaka wasian could still germinate if stored for five months by using plastics bags inside refrigerators. A significant provision of seeds and suitable timing are closely related to the proper seed storage techniques.

Keywords: Seeds, cempaka wasian, storage duration, containers

*TEKNIK PENYIMPANAN BENIH CEMPAKA WASIAN (*Magnolia tsiampaca *(Miq.) Dandy). Cempaka wasian merupakan jenis kayu yang memiliki nilai historis sangat erat dengan budaya masyarakat Minahasa. Pengembangan hutan tanaman cempaka wasian perlu menjadi prioritas mengingat tingkat kebutuhannya cukup tinggi. Penyediaan benih dalam jumlah yang banyak dan pada waktu yang tepat sangat berkaitan dengan usaha metode penyimpanan benih yang sesuai. Penelitian ini bertujuan untuk mengetahui pengaruh waktu, tempat, dan wadah penyimpanan yang sesuai untuk benih cempaka wasian. Rancangan yang digunakan dalam penelitian ini adalah acak lengkap yang disusun dengan pola factorial yang terdiri dari 3 (tiga) faktor, yaitu: 1) waktu penyimpanan, 2) ruang penyimpanan, dan 3) wadah penyimpanan. Waktu penyimpanan terdiri dari 4 faktor, yaitu 2; 3; 4; dan 5 bulan, tempat penyimpanan terdiri dari 3 faktor yaitu ruang kulkas; ruang kamar; dan ruang AC. Sedangkan wadah penyimpanan terdiri dari kain blacu; aluminium foil; dan plastik. Hasil penelitian menunjukkan bahwa benih cempaka wasian masih dapat berkecambah optimal walaupun disimpan selama 5 (lima) bulan apabila menggunakan wadah simpan plastik dalam ruang kulkas.*

*Kata kunci: Benih, cempaka wasian, lama penyimpanan, wadah*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.113-120 

## I. INTRODUCTION

Cempaka wasian is a type of woody tree which has historical value closely associated with the local culture of the Minahasan people. Cempaka wasian timber has been utilized, even before the timber becomes the primary material for Woloan House (traditional house of Minahasa). Lumempouw (2014) stated that in the ceremonial process of building traditional houses in Tombulu (one of the local tribes of Minahasa), cempaka wasian is mostly collected from the forest and the timber is used as the core material. Additionally, the people of Minahasa embrace the psychological relationship with this wood because applying it as the primary house material is believed to provide a daily positive atmosphere.

Currently, cempaka timber supply is limited, and it is collected either from the natural or reserved forest for the traditional houses of Minahasa (Sasmuko, 2010). The development of cempaka wasian plantations must become a priority, due to their considerable consumption and it's culturally significant for the people of Minahasa. It necessitates the supply of a large quantity of cempaka wasian seeds with superior quality. Availability of seeds in large amounts is closely associated with the effort to the proper technique of storing seeds. During storing period, seeds experience a decline in germination value due to their physiological process as well as the surrounding environmental condition which is less optimal (Suita & Darwo, 2015) (Nurisma, Agustiansyah & Kamal, 2015).

Seeds of cempaka wasian are grouped as one of the seeds which cannot be stored for an extended period (Kinho & Mahfudz, 2011). However, little information is recorded on the exact duration and method of storing these seeds. Irawan and Hidayah (2019) address that cempaka wasian seeds which have been stowed for a month in plastic bags were able to grow well with a germination rate of >80%. The experiment to identify longer conservation duration by applying applicable preservation method needs to be studied to complete available records. This study aims to discover the impact of appropriate storage duration, storage rooms, and containers for cempaka wasian seeds.

## II. MATERIAL AND METHOD

The study aims to provide sufficient details of the research work to allow the method to be reproduced. This study was conducted from November 2018 to April 2019 at the Permanent Nursrey of BPDASHL (the Watershed and Protected Forest Management Unit) Tondano Kima Atas located at the office of Environment and Forestry Research and Development Centre, North Sulawesi Province. The material used in this research is the seeds of cempaka wasian, from Minahasa Selatan Regency. Meanwhile, the equipments used was sowing box for sowing seeds, sand, label, digital scale, oven, and stationaries.

### A. Methods

The ripen seeds were extracted from the fruits then soaked overnight to remove the epidermis from the seeds. The seeds are stored according to the treatment. Duration of storage was 2, 3, 4, and 5 months; meanwhile, storage room comprised of the refrigerator (temperature 4–10$^{o}$C; humidity 40–50%), storage chambers with room temperature (temperature 26–30$^{o}$C; humidity 65–80%), and rooms with air conditioners (temperature 18–21$^{o}$C; humidity 51–61%). Further, storage containers consisted of calico cloth, aluminium foil, and plastic bags.

Every treatment comprises of three replications each with 50 seeds. Seed germination was observed for 40 days for each treatment. Seeds were not sown concurrently, but it is adjusted to the treatment of storage duration. The storage duration is grouped for the duration of 2, 3, and 4 months. The seeds were stored for two months, and then it is rejuvenated and observed for 40 days. The treatment of storage duration of 3 months, seeds were stored first for three months then it is rejuvenated and observed for 40 days. The treatment storage duration of 4 months, the seeds were stored for four months; then it was rejuvenated and observed for 40 days. The

treatment of storage duration of five months, the seeds were stored for five months, then it was rejuvenated and observed for 40 days. This study employs a completely randomized design organized with a factorial pattern.

### B. Observed Parameters

Experimental parameters were seed moisture content, germination rate, and germination speed. They were calculated using the following formula:

### Seed Moisture Content

The water level in the seeds was measured using an oven temperature of 103±2°C for 17 hours. Each treatment consisted of 20 seeds. The water content is represented in the percentage of weight and calculated to the closest one decimal (ISTA, 2010) with the following formula:

$$\text{Seed moisture content (\%)} = \frac{(M2 - M3)}{(M2 - M1)} \times 100\% \quad ..(1)$$

Where: M1 = containers' weight before oven (g); M2= containers' weight + seeds before oven (g); M3= containers weight + seeds after oven (g)

### Germination Rate

The energy to grow is determined by the number of seeds which has sprouted. According to Sadjad,Muniarti, and Ilyas (1999) Siahaan (2017), the germination rate lays out the potential viability parameters with the formula:

$$\text{Germination rate (\%)} = \sum \frac{KN}{N} \times 100\% \quad .....(2)$$

where: KN = number of normally germinated seeds; N = number of sown seeds

### Germination Speed

The calculated germination rate is the seeds that germinate from the first observation day to the last day. According to Widajati et al. (2012)) the rate of germination describes the vigour of parameters with the following formula:

$$\text{Germination speed}(\%\ etmal) = \sum_{i=1}^{n} \frac{(KN)i}{Wi} \quad .....(3)$$

where: i=observation day; (KN)i=normal germination on day i (%); Wi= time(etmal) on day i

### C. Analysis

Data were analyzed by using analysis of variance (ANOVA). If the data is significantly different, further testing was carried out using Duncan's analysis.

## III. RESULT AND DISCUSSION

Following the outcome of the analysis of variance, it can be said that the interaction between duration, storage room, and containers treatment offers absolute impact towards seed moisture content and the germination rate of cempaka wasian seeds (Table 1). Further tests to find out the best interaction treatment are shown in Table 2.

Table 1. Analysis of variances of the effect of duration, storage room, and containers treatment towards seed moisture content, the germination rate and germination speed of cempaka wasian seeds

| Source of variation | db | Mean of square | | |
|---|---|---|---|---|
| | | Seed moisture content | Germination rate | Germination speed |
| Duration | 3 | 43.23* | 2,426,41* | 3.09* |
| Room | 2 | 23.40* | 6,320.15* | 11.05* |
| Containers | 2 | 329.60* | 12,176.70* | 21.18* |
| Duration x room | 6 | 2.44* | 129.04* | 0.19* |
| Time x containers | 6 | 12.78* | 840.26* | 1.15* |
| Room x containers | 4 | 72.97* | 1,775.15* | 3.28* |
| Duration x room x containers | 12 | 8.21* | 329.44* | 0.69* |
| Galat (Error) | 35 | 37.64 | 1,753.46 | 0.08 |

Remarks : * = significant at 95% confidence level

Table 2. Duncan test on interaction effect of duration, room, and containers treatment towards seed moisture content, the germination rate and germination speed of cempaka wasian seeds

| No | Treatment | Seed moisture content (%) | Germination rate (%) | Germination speed (% etmal) |
|---|---|---|---|---|
| 1. | Control | - | 88 | 4.67 |
| 2. | 2 month x refrigerator x calico cloth | 9.35 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 3. | 2 month x refrigerator x aluminium foil | 18.12 $^{c}$ | 67.33 $^{a}$ | 2.57 $^{a}$ |
| 4. | 2 month x refrigerator x plastic bags | 20.56 $^{a}$ | 72.00 $^{a}$ | 2.63 $^{a}$ |
| 5. | 2 month x chambers x calico cloth | 11.84 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 6. | 2 month x chambers x aluminium foil | 13.57 $^{e}$ | 14.00 $^{d}$ | 0.45 $^{d}$ |
| 7. | 2 month x chambers x plastic bags | 15.84 $^{cd}$ | 51.33 $^{b}$ | 1.99 $^{b}$ |
| 8. | 2 month x air-conditioned room x calico cloth | 11.67 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 9. | 2 month x air-conditioned room x aluminium foil | 12.58 $^{e}$ | 6.00 $^{e}$ | 1.19 $^{d}$ |
| 10. | 2 month x air-conditioned room x plastic bags | 16.85 $^{cd}$ | 36.67 $^{c}$ | 1.23 $^{e}$ |
| 11. | 3 month x refrigerator x calico cloth | 9.26 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 12. | 3 month x refrigerator x aluminium foil | 11.11 $^{f}$ | 34.67 $^{c}$ | 1.37 $^{c}$ |
| 13. | 3 month x refrigerator x plastic bags | 21.54 $^{a}$ | 74.00 $^{a}$ | 2.81 $^{a}$ |
| 14. | 3 month x chambers x calico cloth | 12.55 $^{ef}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 15. | 3 month x chambers x aluminium foil | 13.15 $^{e}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 16. | 3 month x chambers x plastic bags | 14.59 $^{de}$ | 33.33 $^{c}$ | 1.37 $^{c}$ |
| 17. | 3 month x air-conditioned room x calico cloth | 10.39 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 18. | 3 month x air-conditioned room x aluminium foil | 10.62 $^{f}$ | 2.67 $^{e}$ | 0.10 $^{e}$ |
| 19. | 3 month x air-conditioned room x plastic bags | 18.74 $^{bc}$ | 41.33 $^{c}$ | 1.89 $^{b}$ |
| 20. | 4 month x refrigerator x calico cloth | 8.88 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 21. | 4 month x refrigerator x aluminium foil | 9.77 $^{g}$ | 10.67 $^{d}$ | 0.39 $^{d}$ |
| 22. | 4 month x refrigerator x plastic bags | 20.28 $^{ab}$ | 56.67 $^{b}$ | 2.49 $^{a}$ |
| 23. | 4 month x chambers x calico cloth | 10.48 $^{fg}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 24. | 4 month x chambers x aluminium foil | 11.27 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 25. | 4 month x chambers x plastic | 11.80 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 26. | 4 month x air-conditioned room x calico cloth | 9.42 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 27. | 4 month x air-conditioned room x aluminium foil | 10.36 $^{g}$ | 2.00 $^{e}$ | 0.06 $^{e}$ |
| 28. | 4 month x air-conditioned room x plastic | 12.99 $^{e}$ | 16.67 $^{d}$ | 0.71 $^{d}$ |
| 29. | 5 month x refrigerator x calico cloth | 9.54 $^{g}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 30. | 5 month x refrigerator x aluminium foil | 9.55 $^{g}$ | 10.00 $^{d}$ | 0.41 $^{e}$ |
| 31. | 5 month x refrigerator x plastic bags | 18.90 $^{b}$ | 52.00 $^{b}$ | 2.52 $^{a}$ |
| 32. | 5 month x chambers x calico cloth | 12.41 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 33. | 5 month x chambers x aluminium foil | 12.21 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 34. | 5 month x chambers x plastic bags | 12.60 $^{e}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 35. | 5 month x air-conditioned room x calico cloth | 10.91 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 36. | 5 month x air-conditioned room x aluminium foil | 12.14 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |
| 37. | 5 month x air-conditioned room x plastic bags | 11.86 $^{f}$ | 0.00 $^{e}$ | 0.00 $^{e}$ |

Remarks: Values followed by the same letter are not significantly different at 95% confidence level

Based on Table 2, it can be concluded that the longer the seeds are stowed, the lower the moisture content and the germination energy are. The refrigerator can maintain moisture level and germination energy of cempaka wasian seeds; meanwhile, storage chambers with room temperature and the room with air conditioner decrease them. On the other hand, calico cloth negatively affects the water composite and germination energy of cempaka wasian seeds but will improve if stored in aluminium foil and plastic bags.

Preservation energy is influenced by various factors, such as condition and storage duration (Suszka et al., 2014). Duration is considered to have an actual impact on the percentage

and the speed of seeds' sprouting (Siahaan, 2017; Solikin, 2016; Lestari, 2019). The growth values produced in this research points out that germination of cempaka wasian seeds can be sustained for five months using correct preservation method. Basically, the storage is an exertion to strive for seeds energy to develop optimally by minimalizing seeds' respiration (Suita & Darwo, 2015).

The moisture level of cempaka wasian seeds retained in refrigerators using plastic bags possesses significantly similar values for 3 month conservation duration, whereas, longer storage duration (4 and 5 months) delivers significantly different – lower – values. These values are comparable with germination energy. Seeds which are preserved for 4 and 5 months inside refrigerators using plastic bags attain growth energy of 56.67% and 52% while for 2 and 3 months obtain 74% and 72%. Anto and Jayaram (2010) address that seeds' moisture content positively correlated with the percentage of their germination, loss of seed moisture content will indirectly reduce the proportion of seed germination. Moreover, Mahjabin, Bilal, and Abidi (2015) explained that during the preservation process, water level and temperature are responsible for damaging seeds. Although conserving cempaka wasian seeds by cooling them at temperature 4°-10°C using plastic bags obtained slighter germination energy parallel to storage duration, it is regarded more effective compared to other treatments which experience a dramatic decline in growing capability.

Zhao et al. (2017) stated that storage rooms with low temperatures and dry conditions have a good effect on maintaining the viability of seeds of most species of Hydrocharitaceae and Potamogetonaceae. Besides, reduction in developing ability due to insufficient water level was found in *Pongamia pinnata* (Aminah & Syamsuwida, 2013), *Rhizophora apiculate* (Rohandi & Widyani, 2010) (Nurisma, Agustiansyah & Kamal, 2015) and *Reutealisri sperma* (Tresniawati, Murniati, & Widajati, 2014).

Surya and Normasiwi (2019) stated that the speed of germination refers to the ability of the seeds to germinate in less than optimal environmental conditions. Lesilolo, Patty, and Tetty (2012) added that the speed of seed growth is a process of reactivation of seeds for optimum growth. Growth speed can be expressed as a measure of the duration it takes to achieve an etmal germination. Fast-growing sprouts indicated that the metabolic process is running optimally (Prasetya, Yulianah, Purnamaningsih, 2017, 2017). The speed of germination of cempaka wasian seeds is directly proportional to the germination rate (Tabel 2). Lestari (2019) state that the longer the seeds are stored, the speed of germination or seed growth power will decrease, so that the value of seed germination will also decrease. The value of seed germination is related to the speed of seed germination or seed growth capacity (Payung, Prihatiningtyas, & Nisa, 2012).

Excellent quality and proper packaging can generate good maintenance room ecosystem for seeds as well as sustaining the water level; thus, the seeds can be preserved extensively (Lodong, Tambing, & Adrianton, 2015). Plastic containers can endure the moisture content in the cempaka wasian seeds more productively in comparison to other storage containers because they are water-proof. Debtisari, Erawati, and Sugiyarto (2018) stated that preservation system using water-proof receptacle prevents excessive evaporation within the seeds during the conservation process, and seeds maintained in water-proof storages are capable of sustaining the germination viability and vigour of seeds. Furthermore, several seeds can be well upheld utilizing plastic such as cucumber seeds (Sumpena, 2012), rice seeds (Sari & Faisal, 2017), and *Manglietia glauce* seeds (Suita & Darwo, 2015). Plastic is a packaging substance which is resistant to humidity, pressure, and not fragile (Rahayu & Widajati, 2007).

Furthermore, aluminium foil and calico cloth produce mediocre growth viability values compared to plastics. Cempaka wasian seeds conserved in calico cloth failed to sprout in all storage rooms. This calico cloth is suspected to increase respiration of cempaka wasian seeds; consequently, the moisture content decreases

rapidly. Tuwu, Sutariati and Suaib (2012) indicated that calico cloth has the poorest effects towards the viability of sorghum seeds for the reason that packages made from this material are air translucent; as a result, the exchange between humidity and surrounding air can easily occur.

Additionally, Irawan and Iwanuddin (2019) reveal that calico cloth influences the deterioration water level in *Pometia pinnata* seeds. The seeds of cempaka wasian are assumed to have a higher level of respiration. Suryanto (2013) stated that calico cloth is a material that has large pores so that it is easy for air to pass. This condition causes the seeds to bind or release water into the air easily. This matter can be perceived through the utilization of calico cloth as a means of storage preserved in all three conservation rooms. Seeds preservation through calico textile inside a room with temperature 4°-21°C (room with air conditioner and refrigerator) is generally competent to affect the seeds feasibility compared with no air conditioner room because of the temperature and humidity stability factors.

This situation is comparable to *Manglieta glauca* seeds retained in calico cloth inside room temperature (25°-30°C), which has lower germination capability than preservation inside rooms with air-conditioner and DCS (Suita & Darwo, 2015). Furthermore, Zanzibar (2011) stated that points out that preserving *Pinus merkusii* seeds in room temperature accelerate the rate of seeds' decline. Nurisma, Agustiansyah and Kamal (2015) added that high storage temperature can trigger seeds respiration.

Refrigerators are considered as storage rooms which are proficient at offering optimal effects in storage techniques of cempaka wasian seeds. Seeds packed inside a plastic container in refrigerators significantly maintain the moisture level due to their abilities to avoid greater seed respirations. Purba, Sitepu, and Haryati (2013) explain that respiration occurs because enzyme activity in seeds is determined by moisture content and high relative humidity. Likewise, Taghfir, Anwar, and Kristanto (2018) express that refrigerator temperature conduces enzyme compound in the seeds become less active; hence the respiration process reduces. This deterioration process results in the declension activity of carbohydrate, protein, and seed fat degradation; therefore seed water level relatively unaltered. As Yuniarti and Nurhasybi (2015) expressed that longer drying and preservation will bring about changes in the viability (moisture level and germination energy) and biochemistry contents (fat, carbohydrate, and protein) of *Michelia campaca* seeds on various levels of drying and preserving method. Further, Yuniarti and Djaman (2015) claimed that low storage temperature reduces the speed of seed respiration; hence the period of seed preservation can be extended. Rahayu and Widajati (2007) presented that maintenance of seeds using plastics in refrigerator temperature can endure seeds' vigour of caisim up to 15 weeks. Similarly, Kolo and Tefa (2016) explain that in the cooler temperature respiration develop slowly; thus, the seed viability and vigour can be maintained extensively. Seed quality is sustained by controlling the moisture content during the preservation process inside refrigerators (Lestari, 2019).

Irawan and Hidayah (2019) suggest that if it is observed from the germination rapidity parameter, the stowing condition inside an air-conditioned room using plastic containers is regarded as the ideal circumstance to save cempaka wasian seeds for four weeks (germination rate 85.33%). However, following the findings of this study, it can be said that the condition of the air-conditioned room is insufficient to provide a significant impact on germination energy when stored for 2–5 months. This is due to plastic bags reserved in an air-conditioned chamber adequately supply the effect of optimal condition to cempaka wasian seeds to grow when kept up to 4 weeks. This can be examined from several seeds which have developed in containers when stored using this method. Therefore, in this particular condition, seeds undergo rapid growth. On the contrary, their germination energy will be minimized if held in reserve for more than four weeks because it has reached optimal condition for the seed to develop.

## IV. CONCLUSION

The cempaka wasian seeds are included in the category of seeds that cannot be stored for a long period. Proper storage technique can extend shelf life and not affect its viability. Cempaka wasian seeds can be stored using a plastic container in the refrigerator. In such conditions, cempaka wasian seeds can survive their viability for up to 5 months (germination rate 52%).

## ACKNOWLEDGEMENT

Appreciation is conveyed to the of Enviroment and Forestry Research and Development Institute of Manado, Muchlis, S.Hut.T, MP, the Manager of Permanent Nursery of BPDASHL Tondano Kima Atas, Heri Prayitno, S.Hut, and seedbed officers Eky Kaeng and Opa (Agustinus Pangeke).

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# CHEMICAL AND ORGANOLEPTIC PROPERTIES OF BEKAI (*Pycnarrhena tumefacta* Miers) LEAVES FOR FLAVOURING AGENT (BIO-VETSIN)

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Received: 17 April 2020, Revised: 26 October 2020, Accepted: 27 October 2020

CHEMICAL AND ORGANOLEPTIC PROPERTIES OF BEKAI (*Pycnarrhena tumefacta* Miers) LEAVES FOR FLAVOURING AGENT (BIO-VETSIN). The "tasty" cuisine tends to use chemical flavour agent containing monosodium glutamate (MSG). Utilization of MSG, in long-term, may cause health problems, especially triggering cancer cells. Therefore, it is necessary to introduce and increase a natural flavouring agent to eliminate those health problem, such as bekai leaf. Bekai (*Pycnarrhena tumefacta* Miers) is familiar as a natural flavour agent (bio-vetsin) in cuisine for forest communities in Nyapa Indah Village, Berau, East Kalimantan, Indonesia. However, until now there has been no proper analysis support for widespread utilization of bekai leaf. This paper studies the presence of phytochemicals, antioxidant and GC MS analysis from bekai leaf extracts, as well as five hedonic classifications of organoleptic test, to reinforce the need for a better understanding of consumers reaction in terms of possible acceptance of additional bekai leaves applied in soup as bio-vetsin. Present study showed that the qualitative screening of phytochemical compounds in bekai leaves ethanolic extracts revealed presence of alkaloids, flavonoids, tannins and steroids. Antioxidants of bekai leaves using 2,2-diphenyl-1-picrylhydrazy (DPPH) method showed that concentrated extract has 80.1%, which was predicted can improve immune for inhibitory action of cancer cells. GC MS analysis suspected that bekai leaf extract contained five major compounds, i.e. oxirane dodecyl, gamma sitosterol, vitamin E (α tokoferol), 9.12-Octadecadienoic acid (Z,Z)- (natural linoleic acid), and 3-Tetradecanynoic acid (myristic acid). These chemical compound in related with their phytochemical were predicted to contain strong antioxidant activities and some of them are commonly used as flavour agent in cuisine for some food industries. Meanwhile, results of organoleptic tests presence in three soup variant showed that soup with additional bekai leaves has best acceptance in the children's perception due to unique smell, tasty and no colour changing compared with added MSG and control. Thus bekai leaf can be used as an innovation for healthy food and new market opportunities for MSG substitutes.

Keywords: Bekai leaf, phytochemicals, antioxidants, GC-MS, flavouring agent, organoleptic

*KARAKTERISTIK KIMIA DAN ORGANOLEPTIK DAUN BEKAI (*Pycnarrhena tumefacta *Miers) UNTUK PENYEDAP ALAMI (BIO-VETSIN). Masakan "enak" cenderung menggunakan bahan penyedap kimia yang mengandung monosodium glutamat (MSG). Pemanfaatan MSG dalam jangka panjang dapat menyebabkan gangguan kesehatan, terutama pemicu sel kanker. Oleh karena itu perlu dilakukan pengenalan dan penambahan zat penyedap alami untuk mengatasi gangguan kesehatan tersebut, seperti daun bekai. Bekai (*Pycnarrhena tumefacta *Miers) dikenal sebagai penyedap alami (bio-vetsin) dalam masakan oleh masyarakat di Desa Nyapa Indah, Berau, Kalimantan Timur, Indonesia. Namun hingga saat ini belum ada dukungan analisis yang tepat untuk pemanfaatan daun bekai secara luas. Penelitian ini menentukan keberadaan fitokimia, anti oksidan dan analisis GC MS dari ekstrak daun bekai, serta lima klasifikasi hedonik uji organoleptik, untuk memperkuat perlunya pemahaman yang lebih baik tentang reaksi konsumen dalam hal kemungkinan penerimaan penyedap asal daun bekai yang ditambahkan dalam sup sebagai bio-vetsin. Penelitian terbaru*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.121-133 

*menunjukkan bahwa skrining kualitatif senyawa fitokimia pada ekstrak etanol daun bekai menunjukkan adanya alkaloid, flavonoid, tanin dan steroid. Anti oksidan daun bekai dengan metode 2,2-difenil-1-pikrilhidrol (DPPH) menunjukkan ekstrak pekat memiliki kandungan 80,1% yang diperkirakan dapat meningkatkan kekebalan tubuh untuk menghambat aksi sel kanker. Analisis GC MS diduga ekstrak daun bekai mengandung 5 (lima) senyawa utama yaitu oksiran dodesil, gamma sitosterol, vitamin E (a tokoferol), 9.12-oktadekadienoat (Z, Z) - (asam linoleat alami), dan 3-Tetradekanynoat (asam miristat). Senyawa kimia yang terkait dengan fitokimianya diduga mengandung aktivitas antioksidan yang kuat dan beberapa di antaranya biasa digunakan sebagai agen penyedap masakan pada beberapa industri makanan. Sedangkan dari hasil uji organoleptik keberadaan tiga varian soto menunjukkan bahwa dengan tambahan daun bekai paling diterima persepsi anak-anak karena memiliki bau yang khas, gurih dan tidak berubah warna dibandingkan dengan penambahan MSG dan kontrol. Dengan demikian daun bekai dapat digunakan sebagai inovasi untuk makanan sehat dan peluang pasar baru untuk pengganti MSG.*

*Kata kunci: Daun bekai, phytochemical, anti-oksidan, GC-MS, penyedap alami, organoleptik*

## I. INTRODUCTION

People choose high quality food that provide health benefits by adding certain ingredients (additives) in food (Sauceda, Martinez, Rodriguez, Aguilar, & Zavalal, 2016). An increase in economic rate raises the population's awareness of food additives usages (Kumar, Singh, Chandra & Samsher, 2017). These additive materials have begun to shift from factory synthesis materials, such as MSG (Monosodium glutamate) to natural ingredients. This is because to MSG utilizations, in long-term, can become a toxic and possibly threat to public health, especially triggering cancer cells (Niaz, Zaplatic & Spoor, 2018).

Indonesia is one of the countries with mega-biodiversity and some plants are potentially used for food spices and herbs (Supartono, Sukartiko, Yuliando, & Kristanti, 2015). Some spices and herbs are known to be used as cuisine ingredients such as ginger and galangal. Ginger (*Zingiber officinale*) is extensively used around the world in cuisine as a spice (Ghosh, Banerjee, Mullick, & Banerjee, 2011). While, *Alpinia galanga* (galangal) is rhizome used as spice and flavouring agent and its leaves and inflorescence are consumed as vegetable (Wong, Lim, & Omar, 2008). There are many other potentials of herbal plants in Indonesia that has a function as natural flavouring agent and can be used as an MSG substitute.

One of the potential herbaceous plants as natural flavouring agent is bekai (*Pycnarrhena tumefacta* Miers) from Menispermaceae family. Hereditary, local community of Nyapa Indah Village, Berau District, East Kalimantan, Indonesia planted bekai both on local yard and under forest tree stands (Figure 1). Usually bekai leaves are added into cuisine which meat as basic ingredients, while for other cuisine has never been tried. Besides for accelerating process of meat into well-done, bekai leaves could also be used as natural flavouring agent. However, there has been no research publication available on the content of chemical compounds and also organoleptic tests on cuisine or food with additional bekai leaves (*Pycnarrhena tumefacta* Miers). As a comparison in other genera with different species, namely sengkubak (*Pycnarrhena cauliflora* (Miers) Diels) in West Kalimantan, the community have used it as a basic ingredient for accelerating process of meat and savoury flavours in food. *Pycnarrhena cauliflora* (Miers) Diels has roots that are predicted to play a role as cytotoxic and pro-apotic activities in human breast cancer (Masriani, Mustofa, Jumina, Sunarti, & Enawaty, 2014). Further, Masriani, Mustofa, Jumina and Sunarti (2013) stated that the ethanolic extracts from root, stem and leaves of *P. cauliflora* inhibited the growth of HeLa cells (cervical cancer cell).

Development of novel foods by adding functional component to carrier food provides new market opportunities for manufacturers (Pestoric et al., 2015). In order to be successful on the market a product or product category

needs to both benefit from positive general image and offer product qualities that match or surpass consumers' expectations (Almli, Verbeke, Vanhonacker, Naes, & Hersleth, 2011). Thus, a considerable proportion of product failure can be attributed to a mismatch between sensory properties and consumer needs or expectations (Kemp, Hollowood, & Hort, 2009). The positive general image of consumer through human senses include colour, odour, taste, and others special feature were necessary to maintain reproducible efficacy and safety of herbal product as rational drug (Bisla, Choudhary, & Chaudhary, 2014).

In the present study, some chemical properties of bekai leaves were determined as a natural flavouring agent (bio-vetsin), especially for others cuisine (i.e. soup). This study also provided organoleptic data of its cuisine products to reinforce the need for a better understanding of consumers reaction in terms of possible acceptance of additional bekai leaves as an innovation for healthy food and new market opportunities for MSG substitutes.

## II. MATERIAL AND METHOD

### A. Materials

Fresh leaves of bekai (*Pycnarrhena tumefacta* Miers) were collected from Custom Forest of Nyapa Indah Village (around Labanan Forest Research), Berau District, East Kalimantan, Indonesia (N 01°52 '48.2 "and E 117 ° 18' 03.2"). Bekai leaves were dried in the laboratory with air conditioned (A.C.) set for 25°C for 3 days and ground to a fine powder using crusher and sieved through 40–60 mesh. The powder samples were kept at A.C. room in a covered glass container to protect them from humidity and light prior to extraction. Then these samples were prepared for further analysis.

### B. Methods

#### 1. Maceration

Five grams dried powder of bekai leaves were exhaustively extracted by maceration in 200 ml ethanolic solvent for 24 hours at room temperature (28+2°C). Whereas, each extraction was concentrated from 200 ml into 10 ml concentrated crude ethanolic extracts, dried in oven at 50°C to give dark green extracts (Maharani et al., 2016; Zhang et al., 2018). Further, these extracts were used for phytochemical and antioxidant analysis (5 ml), and GC-MS analysis (5 ml).

#### 2. Antioxidant Assay

In this antioxidant test, 100% of 5 concentrated samples were grouped into 200 ppm, 100 ppm, 50 ppm and 25 ppm times of dilution, respectively. Further, 1 mg of vitamin C was weighed, then dissolved in 5000 µl of

(a) (b)

Figure 1. Bekai planted in forest community area (a), and bekai leaf used for natural flavouring agent (b)

distilled water and regarded as a positive control. While, negative control was used its solvent (distilled water) (Fitriana, Istiqomah, Ersam, & Fatmawati, 2018), 100 µl sample was mixed in cuvette with 400 µl of distilled water was added, and 500 µl of 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging activity. Mixing was stopped when the sample volume has reached 1000 µl (1 ml). Samples were incubated for 20 minutes indoor with minimum light. The antioxidant activity was determined by decolorization of DPPH with a wavelength of 517 nm using a spectrophotometer. The scavenging activity was calculated as a percentage of DPPH decolouration relative to a negative control using equation 1 (Maheswari, Reena, & Sivaraj, 2017).

**C. Analysis**

**1. Preliminary Phytochemical Analysis**

Bekai extracts were tested for active compound such as flavonoids, saponins, steroids, tannins, terpenoids, alkaloids and carbohydrate using some following standard procedures (Keo et al., 2017; Jaradat, Hussen, & Ali, 2015).

**2. Flavonoids Determination**

About 1 ml of ethanolic extract was mixed and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonical layer (bottom layer) indicates the presence of flavonoids (Jaradat et al., 2015).

**3. Saponins Determination**

Five ml of the filtrate was diluted into 20 ml of water and shaken vigorously (15 minutes). A stable froth (foam) upon standing indicates the presence of saponins (Keo et al., 2017).

**Steroids Determination**

One ml of ethanolic extract of each sample is boiled with 10 ml of chloroform and it was cooled accordingly. Then, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. The mixtures were then shook well and it was allowed to stand for some time. The red color appears at the lower layer indicates the presence of steroids (Keo et al., 2017).

**4. Tannins Determination**

Test solution of 5 ml ethanolic extract with 1% sodium hydroxide solution (1%) provides yellow to red precipitation within short time indicates the presence of tannins (Keo et al., 2017).

**5. Triterpenoid Determination**

One ml of ethanolic extract of each sample is boiled in the mixture of 10 ml chloroform and cooled down. One to two drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake tube well and allow standing for some time, reddish-purple colour appears at the lower layer indicates the presence of Triterpenoids (Keo et al., 2017).

**6. Alkaloids Determination**

Five ml of ethanolic extract was reacted with two drops of potassium bismuth iodide solution reagents in test-tubes. The development of creamy and orange colour respectively indicated positive result (Keo et al., 2017).

**7. Carbohydrate Determination**

The extract was hydrolyzed with HCl in the water heater. Then, it was added with 1 ml of pyridine and a few drops of sodium nitroprusside solution into the hydrolyzate, after it was etched with an alkaline solution of sodium hydroxide. The formation of pink to red colour indicates the presence of glycosides (Keo et al., 2017).

**8. Gas Chromatography-Mass Spectrometry Analysis (GC-MS Analysis)**

Gas Chromatography-Mass Spectrometry (G.C.–M.S.) analysis was carried out for the ethanolic extracts. The analysis was performed according to the GC-MS equipments by Shimadzu Q.P. 2010: R.T.X. - column type is 5 ms, Restek Corp (30 m length). The injector and detector temperatures were both maintained at 250°C, while operation temperature was set at 50–300°C. The column temperature was programmed at 50–120°C, with 40°C increment per minute which was maintained

for one minute. Then, it was programmed at 120–300°C, with 60°C increment per min and held for five minutes, with retention time (Rt) of 60 minutes. Helium was used as a carrier gas is 50–500 atomic mass unit (A.M.U.). The compounds of each extract were identified by using computer searchers in commercial libraries of NIST (Maharani, Fernandes, Turjaman, Lukmandaru & Kuspradini, 2016). Furthermore, the structure of chemical compounds associated with phytochemical and antioxidant tests determined will be drawn by using chemical office software.

**9. Organoleptic or Macroscopic Evaluation Analysis**

Based on observing, touching and sniffing senses, organoleptic analysis was carried out by a human panel (Xu et al., 2018). In this study, organoleptic analysis were conducted by comparing soup with bekai leaves addition (9 airdried leaves for 2 L), soup with MSG addition (half-one teaspoon for 2 L), and soup without any additional flavour (as a control). Organoleptic analysis of soup were carried out on 20 children in average 10-12 years old as panelist one by one by using Hedonic scale with 5 classifications (very dislike=1, dislike=2, neutral=3, like=4, and very like=5). They were decided on their favorite soup for sensory attributes of odor, flavour/taste and color.

## III. RESULT AND DISCUSSION

### A. Phytochemical Test of Bekai Leaf

Qualitative screening of bekai (*Pycnarrhena tumefacta* Miers) leaves ethanolic phytochemical compounds showed the presence of alkaloids, flavonoids, tannins and steroids. Previous study of other Pycnarrhena genus stated that *Pycnarrhena longifolia* leaves contained alkaloid (Masriani et al., 2013) and flavonoids (Mohammed et al., 2020). Meanwhile, others *Pycnarrhena manillensis* included Philippine endemic medicinal plant, it leaves contained alkaloids and steroids (Ragasa, Tepora, & Rideout, 2009). Another genus from West Kalimantan, Indonesia known locally as sengkubak root (*Pycnarrhena cauliflora*) has been determined for its alkaloid content which is identified as an anticancer compound (Masriani et al., 2014).

### B. Antioxidant Test of Bekai Leaf

The result of the antioxidant test of bekai leaf using 2,2-diphenyl-1-picrylhydrazy (DPPH) method showed that concentrated extract had DPPH absorption reduction of 44.5% in 200 ppm dilution, 68.1% in 100 ppm dilution, 80.1% in 50 ppm dilution, and 83.2% in 25 ppm dilution (Table 1). DPPH is used to test the ability of the compound to act as a free radical trap or hydrogen donor and evaluate the antioxidant activity (Devi & Ganjewala, 2011). Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems (Pirbalouti, Firoznezhad, Craker, & Akbarzadeh, 2013).

Antioxidant tested of bekai leaves using DPPH method showed that concentrated extract was 80.1%, almost equivalent to high dose of Vitamin C of same genus, Tubu (*Pycnarrhena longifolia*) leaves that it had 87% antioxidant activity (Mohammed et al., 2020). The interpolation was calculated from 0 (zero) to the optimum concentration, which is between 0 - 83.2%. The optimum reading of antioxidants is at a concentration of 25 ppm. As comparison, vitamin C 100 ppm has an antioxidant of 97.1% and 96.1% in 50 ppm. The colour of sample can affected reading, a high concentration of sample colour is getting stronger or darker. In this study, bekai samples had a dark green blackest extracted colour. Sometimes the sample that containing phenolic

Table 1. Antioxidant test of bekai leaf with DPPH method

| | 200 ppm | 100 ppm | 50 ppm | 25 ppm |
|---|---|---|---|---|
| Bekai (%) | 44.5 | 68.1 | 80.1 | 83.2 |
| Vit C (%) | - | 97.1 | 96.1 | - |

group such as anthocyanin, could have colour interference of the DPPH assay. The samples leads to under-estimation of antioxidant activity (Choong et al., 2007). Other study stated that a limitation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability (DRSA) due to the presence of pigments and colours in the extracts of plant-based foods was addressed. This fact could interferes the elaborate absorbance readings of DPPH radicals (Yeo & Shahidi, 2019).

The high value of antioxidant of bekai leaves could improve human immune for inhibitory action of cancer cells, as well as almost 47% of anticancer drugs, come from the natural product (Jabeen, Hanif, Khan, & Qadri, 2014). Consuming of foods rich in natural antioxidants, as well as processed foods enriched with them, provides the desired supply antioxidant and prevent potential diseases (Hardy, 2000; Jukic, Hmjica, & Aldžic, 2015). Meanwhile, the evaluation of antioxidants with complex compounds are cannot be done singly. The DPPH method is based solely on removal of electrons which are replaced by hydrogen atoms from DPPH and is influenced by the polarity of compound (Gangwar et al., 2014).

### C. A Chemical Compound in Bekai Leaves Based on GC-MS Test

The GC-MS test results (Figure 2, Table 2) indicated that the ethanol leaf extract of bekai contains five major compounds, ie 17.1% Oxirane dodecyl, 14.1% gamma sitosterol, 11.3% vitamin E (α tokoferol), 10.7% 9.12-Octadecadienoic acid (Z,Z)- (natural linoleic acid), 7.5% 3-Tetradecanynoic acid (myristic acid). As well as major compound in bekai leaf, Gazzola (2016) stated that sodium dodecyl sulphate contained in fresh leaves of some natural products have been significant source of anticancer agents because of its antioxidant activity.

According to phytochemical tests, bekai leaves contained flavonoids, which are predicted to be able to provide various flavours in many food (Tanwar & Modgil, 2012). Flavonoids belong to low molecular weight with phenolic compounds (Panche, Diwan, & Chandra, 2016). Strong antioxidant activities in medicinal plants are due to the presence of phenolic compounds (Karau, Njagi, Machocho, Wangai, & Nthinga, 2015). Flavonoids in bekai leaves was Phenol, 2,4-bis(1,1-dimethyl ethyl)-. Phenol, 2,4-bis(1,1-dimethyl ethyl)- ($C_{14}H_{22}O$) detected in the third peak, 2.0% (Figure 3.). These components have same antioxidant properties as the major compound, oxiran dodexyl, which has flavouring agent properties due to include in same dodecyl group. This group is included in the list of chemical flavouring substances allowed for manufacturing of edible flavours (Customs Union Commission, 2011).

Bekai leaves were also contained alkaloids based on the phytochemical test. The alkaloid in food represented the bitter gustatory sensation (Astray, Rio, Mejuto, & Pastrana , 2007). Alkaloids are low molecular weight, and nitrogen-containing compound (Matsuura & Neto, 2015). Alkaloids in bekai leaves were

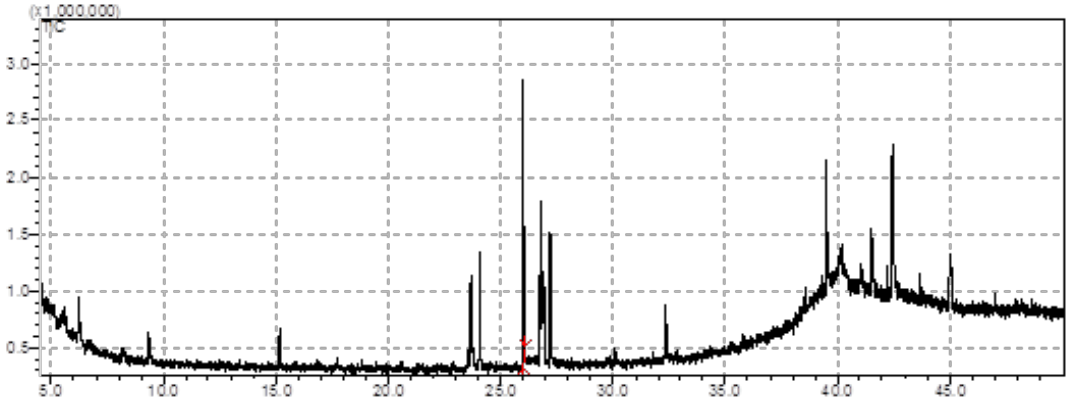
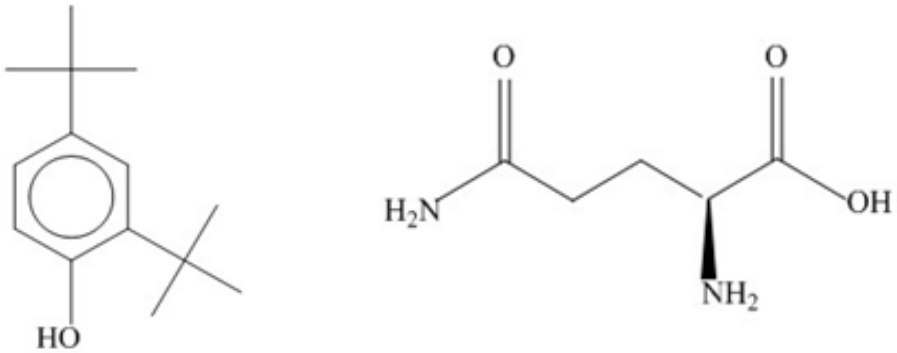



Figure 2. GC-MS analysis of bekai leaves

Table 2. A chemical compound of bekai leaves extract based on GC-MS test result

| Peak# | Ret.Time | Name | Mol.Form | Area (%) | Similarity index (%) |
|---|---|---|---|---|---|
| 1 | 6.231 | Nonane, 2-methyl- | $C_{10}H_{22}$ | 2.8 | 90 |
| 2 | 9.349 | Glutamine | $C_5H_{10}N_2O_3$ | 1.1 | 93 |
| 3 | 15.166 | Phenol, 2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 2.0 | 91 |
| 4 | 23.681 | Phthalic acid, dibutyl ester | $C_{16}H_{22}O_4$ | 5.3 | 91 |
| 5 | 24.062 | Palmitic acid, ethyl ester | $C_{18}H_{36}O_2$ | 6.2 | 96 |
| 6 | 26.023 | Oxirane, dodecyl- | $C_{14}H_{28}O$ | 17.1 | 92 |
| 7 | 26.784 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{21}H_{38}O_4$ | 10.7 | 95 |
| 8 | 26.86 | 9-Octadecenoic acid, ethyl ester | $C_{20}H_{38}O_2$ | 4.2 | 94 |
| 9 | 26.899 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- | $C_{20}H_{34}O_2$ | 4.2 | 91 |
| 10 | 27.228 | 3-Tetradecanynoic acid | $C_{14}H_{24}O_2$ | 7.5 | 90 |
| 11 | 32.357 | Phthalic acid, mono-(2-ethylhexyl) ester | $C_{16}H_{22}O_4$ | 3.5 | 90 |
| 12 | 39.521 | Vitamin E (alpha tocopherol) | $C_{29}H_{50}O_2$ | 11.3 | 96 |
| 13 | 41.526 | Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2-dimethylpropyl-2-methylcyclopropyl]- | $C_{30}H_{50}O$ | 4.7 | 90 |
| 14 | 42.465 | .gamma.-Sitosterol | $C_{29}H_{50}O$ | 14.1 | 97 |
| 15 | 45.018 | 9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)- | $C_{31}H_{52}O$ | 5.4 | 90 |

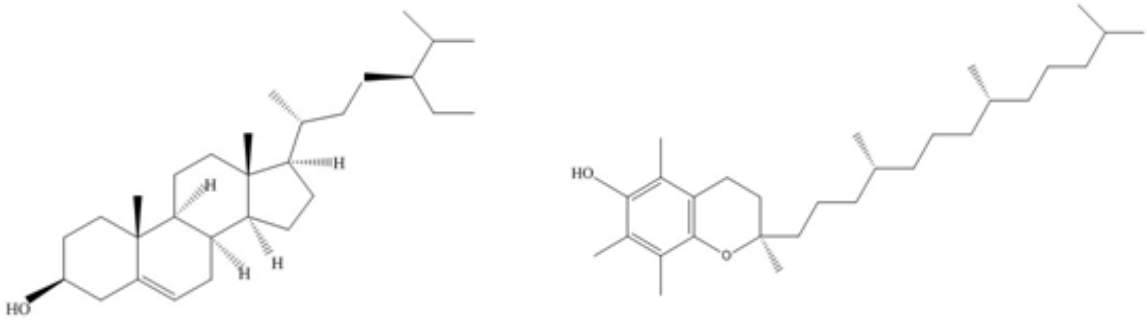



Phenol, 2,4-bis(1,1-dimethylethyl)- Glutamine

Figure 3. Phenol, 2,4-bis(1,1-dimethyl ethyl)- and glutamine suspected in fresh leaves of some natural products, as well as bekai leaves

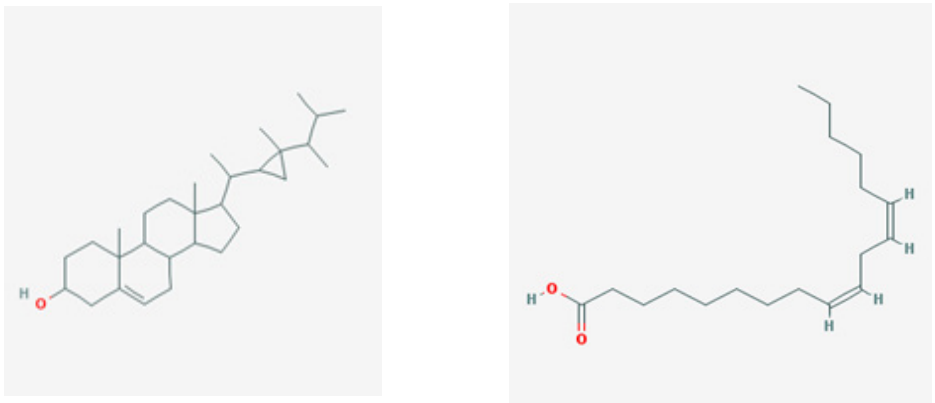
identified as glutamine ($C_5H_{10}N_2O_3$). Glutamine detected in the second peak, 1.1%. Glutamine is a conditionally essential amino acid widely used in sports nutrition due to $NH_2$ ($NH_2$ functional group) presences (Coqueiro et al., 2019). Furthermore, glutamine from snakehead fish could be increased glutathione, which has antioxidant activity in the human body and brain (Sunarno, 2015).

Steroids contained in bekai leaves were gamma sitosterol and α-tocopherol (Figure 4.). α-tocopherol detected in the 12$^{th}$ peak, 11.3% considered to be one of the most powerful antioxidants (Fritsche, Wang, & Jung, 2017). Kuchan et al. (2018) determined that α-tocopherol plays an important role in protecting cell membrane. Meanwhile, gamma sitosterol detected in the 14th peak, 14.1%, and



gamma sitosterol α-tocopherol

Figure 4. Gamma sitosterol and vitamin E (α tocoferol) suspected in fresh leaves of some natural products, as well as bekai leaves



Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2 dimethylpropyl-2-methylcyclopropyl]- Linoleic acid

Figure 5. Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2 dimethylpropyl-2-methylcyclopropyl]- and linoleic acid suspected in fresh leaves of some natural products, as well as bekai leaves

it can be considered as hypolipidemic agent (Balamurugan, Stalin, Aravinthan, & Kim, 2014) and antihyperglicemyc activity (Sirikhansaeng, Tanee, Sudmoon, & Chaveerach, 2017).

In addition, other chemical compounds that are included in the steroid group and contained in bekai leaves were Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2 dimethylpropyl-2-methylcyclopropyl]- or known as gorgosterol detected in the 13th peak, 4.69% (Figure 5.). Pregn-5-en-3.beta.-ol, 20.alpha.-[(1R,2R)-2-(1R)-1,2 dimethylpropyl-2-methylcyclopropyl]- that belong to pregnen compound. Cheenpracha et al. (2017) stated that Pregnen compound belong to the steroid group due to has a C-cyclic bond that showed its strong antioxidant activities.

It can be shown in Table 2, some fatty acids presented linoleic and myristic acid (9.12-Octadecadienoic acid (Z,Z)- (natural linoleic acid) detected in the 7th peak, 10.7%). Linoleic acid consumption can be used as optimal dietary human health (Jandacek, 2017). Linoleic acid has antioxidant activities, including free radical scavenging capacity (Ali et al, 2012). 3-Tetradecanynoic acid (myristic acid) detected in 10th peak, 7.49%. Myristic acid can be used as flavour agent in cuisine and commonly used in some food industries (Burdock & Carabin, 2007). Myristic acid has antioxidant activities in vitro and hepatoprotective effects against carbon tetrachloride-induced acute liver injury (Liu, Yuan, Ramaswamy, Ren, & Ren, 2019).

On the other hand, esters were major components of flavour widely distributed in nature (Bayout et al., 2019). Bekai leaves contained esters, i.e. palmitic acid ethyl ester and 9-Octadecenoic acid, ethyl ester. Palmitic acid ethyl ester detected in the 5th peak, 6.2% (Table 2). Palmitic acid ethyl ester known as hexadecanoic acid ethyl ester, was reported as flavour activity (Gideon, 2015). Other biological activities were antioxidant, hypercholesterolemic, nematicide, pesticide, antiandrogenic flavour, haemolytic and alpha-reductase inhibitor (Sudha, Chidambarampillai, & Mohan, 2013). 9-Octadecenoic acid, ethyl ester detected in the 8th, 4.17%. 9-Octadecenoic acid, ethyl ester had biological activity as hepatoprotective, anti-histaminic, antieczemic and hypocholesterolemic (Arora, Kumar, & Meena, 2017).

### D. Organoleptic Test of Cuisine (soup) with Additional of Bekai Leaves Compared with MSG

In order to maintain quality, purity, potency, safety, and efficacy of herbal drugs or product of medicinal plant needs sensory/organoleptic analysis as consumer acceptance (Patil et al., 2013; Vanhonacker et al., 2013). A sensory experience is described as an individual's perception of goods or services or other essentials in a service process as an image that challenges the human mind and senses. Thus, this sensory marketing is defined as a way of measuring and explaining consumer emotions as well as spotting and capitalizing on new market opportunities, and finally ensuring long-lasting product success (Randhir et al., 2016).

Results of organoleptic tests presence in three soup variants can be shown in Table 3, as flavour agent for children's a flavour/taste perception.

Generally, most children prefered best (very like) in taste, colour and odour of soup with an additional of bekai leaves compared to MSG added and control (Table 3). Soup with an additional of bekai leaves was offered a unique odour/scent, savory/tasty, and no colour changing compared to both MSG added and control. A unique odour/scent in soup with an additional of bekai leaves described fresh scent and very tempting to taste it. Our sense of smell guards us safe by serving us to choose fresh food and avoid rotten/bad food. Various readings have considered the appeal of scents arising from an object, or a service associated with it being perceived as pleasant or unpleasant,

Table 3. Comparison of organoleptic tests presence in soup with addition of bekai leaf, MSG, and without both additional (control)

| Soup Variant | Class | Odour | Flavour | Colour | Remarks |
|---|---|---|---|---|---|
| Soup with bekai leaves | 1 | 0 | 0 | 0 | A unique smell/scent, savoury/ tasty, and no colour changing |
| | 2 | 0 | 0 | 0 | |
| | 3 | 2 | 0 | 3 | |
| | 4 | 7 | 4 | 2 | |
| | 5 | 11 | 16 | 15 | |
| Soup with MSG | 1 | 0 | 0 | 0 | As usual/tasty, and more colouring |
| | 2 | 0 | 0 | 0 | |
| | 3 | 4 | 0 | 7 | |
| | 4 | 6 | 7 | 2 | |
| | 5 | 10 | 13 | 11 | |
| Soup without bekai and MSG (control) | 1 | 0 | 0 | 0 | As usual/commonly tasty, and no colour changing |
| | 2 | 0 | 0 | 0 | |
| | 3 | 3 | 3 | 7 | |
| | 4 | 15 | 14 | 11 | |
| | 5 | 2 | 3 | 2 | |

Remarks : - 20 children in average 10-12 years old ; - Hedonic scale with 5 classifications (very dislike=1, dislike=2, neutral=3, like=4, and very like=5)

and also establishes a positive perception of a particular commercial environment (Bone & Ellen, 1999; Chebat & Michon, 2003).

The flavour or taste sense is the most important and most developed of all senses (Randhir et al., 2016). Organoleptic tests showed that the soup with the an addition of bekai leaves had a fresher, savory, and slightly sweet taste, when viewed from the children's perceptions as panelists who really like and have experience tasting in various types of soup. Furthermore, Randhir et al. (2016) stated that very few of our taste preferences are biologically preset. Much rather they are linked with some sort of experience. Once a flavour or food is accepted, this can also influence the preference for and acceptance of new flavours or foods. Another example of natural substance that could improve organoleptic test results is Mentha arvensis. The use of Mentha arvensis extract on whet-based pineapple mint beverages could improve the colour, taste, appearance and acceptability of the respondents (Kumar et al., 2017).

## IV. CONCLUSION

A recent study showed that the qualitative screening of phytochemical compounds in bekai leaves ethanolic extracts revealed the presence of alkaloids, flavonoids, tannins and steroids. Antioxidants of bekai leaves using DPPH method showed high activity 68.1% in 100 ppm, 80.1% in 50 ppm dilution, and 83.2% in 25 ppm dilution, respectively. Ethanol leaf extract of bekai contained five major compounds, i.e. Oxirane dodecyl, gamma sitosterol, vitamin E (α tokoferol), 9.12-Octadecadienoic acid (Z, Z)-(natural linoleic acid), and 3-Tetradecanynoic acid (myristic acid). Bekai leaves were considered to be one of the powerful antioxidants which can be used as a flavouring agent in cuisine and commonly used in some food industries.

Results of organoleptic (hedonic) tests presence in soup with bekai leaves added has been provided a unique smell, tasty without changing in original colour of soup compared with additional MSG and or control. It can be proved that soup with bekai leaves has been a preference for children's taste perceptions, even though the current generation is very comfortable and accustomed to adding MSG to their consumed food. Thus, this study provided an innovative product of herbal medicine served in cuisine (soup) that it would be predicted to attract consumers to the new market opportunities, and to promote general positive image for early generation consumers to prefer more healthy food.

## ACKNOWLEDGEMENT

The authors want to express our sincere gratitude to Mr Amiril Saridan (taxonomist) for his contributions to this paper explicitly and intellectually. Sincere gratitude also goes to forest communities in Nyapa Indah Village, Berau, East Kalimantan for their beneficial assistances and valuable information related to this research undertaking until its completion.

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# A COMPREHENSIVE INVENTORY AND ECOLOGICAL ASSESSMENT OF ALIEN PLANT INVASION IN MIZORAM, INDIA

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Received: 21 April 2020, Revised: 10 October 2020, Accepted: 27 October 2020

A COMPREHENSIVE INVENTORY AND ECOLOGICAL ASSESSMENT OF ALIEN PLANT INVASION IN MIZORAM, INDIA. Invasion by alien plants has a detrimental effect on the natural forest plant community, leading to the loss of native species. An inventory of alien plants facilitates in determining the potential threats to the natural plant biodiversity. This present paper evaluates the alien plants in Mizoram (an Indo-Burma hotspot region) and provides the first authentic inventory of alien plants of Mizoram along with their diversity, ecological aspects, origin, and status of invasion. Extensive field surveys were done during July 2018 to September 2019 in different protected areas of Mizoram. We adopted Random Sampling Technique using nested quadrats in a plot size of 400 $m^2$ (20 m × 20 m), within which a quadrate size of 5 m × 5 m was laid for shrubs and 1 m × 1 m was laid for herbs. Plant specimens were collected, dried, poisoned with 0.1% Mercuric Chloride ($MgCl_2$); voucher specimens were prepared and deposited in Central National Herbarium, Botanical Survey of India (CAL) after proper identification.The results revealed the occurrence of 163 alien plant species under 135 genera belonging to 51 families in Mizoram. Maximum species were represented by family Asteraceae with 29 species followed by Leguminosae (19 species), Convolvulaceae (9 species), Euphorbiaceae (7 species) and Amaranthaceae (7 species). The 57.66% of the alien species reported from Mizoram were of American origin followed by 11.65 % from African, 7.36% from Mexican and 5.52% from Australian origin. Life form analysis revealed the presence of 58.64 % herbs, 15.43 % trees and 9.87% shrubs. Out of the whole alien plants recorded, 91 species were used for traditional medicines, 43 species as ornamental, 15 species were edibles, nine species used as timber and four species used as green manure. The study also recommends the ten most obnoxious species, five neo-invasive plants, which have the greatest potential threats to the native flora. The process and probable causes of invasion in the state were also discussed briefly, which may be utilized in the preparation of conservation or forest management policies.

Keywords: Alien plants, invasive, neo-invasive, biodiversity, Mizoram

*INVENTARISASI KOMPREHENSIF DAN PENILAIAN EKOLOGI INVASI JENIS-JENIS ASING DI MIZORAM, INDIA. Invasi oleh tumbuhan asing memiliki efek yang merugikan pada komunitas tumbuhan hutan alam, yang menyebabkan hilangnya spesies asli. Inventarisasi tumbuhan asing memudahkan untuk menentukan potensi ancaman terhadap keanekaragaman hayati tumbuhan alami. Tulisan ini mengevaluasi tumbuhan asing di Mizoram (wilayah hotspot Indo-Burma) dan merupakan inventarisasi otentik pertama tanaman asing Mizoram bersama dengan keanekaragaman, aspek ekologi, asal-usul dan status invasi mereka. Survei lapangan ekstensif dilakukan dari Juli 2018 hingga September 2019 di berbagai kawasan lindung Mizoram. Pengambilan sampel mengadopsi Teknik Pengambilan Sampel Acak menggunakan kuadrat bersarang di plot berukuran 400 $m^2$ (20 m x 20 m), di mana ukuran kuadrat 5 m x 5 m diletakkan untuk semak dan 1 m x 1 m untuk tumbuhan. Spesimen tanaman dikumpulkan, dikeringkan, diracun dengan 0,1% Mercuric Chloride ($MgCl_2$); voucher spesimen disiapkan dan disimpan di Central National Herbarium, Botanical Survey of India (CAL) setelah identifikasi yang tepat. Hasil penelitian menunjukkan terjadinya invasi 163 spesies tumbuhan asing di bawah 135 genera milik 51 famili di Mizoram. Spesies terbesar yang teridentifikasi adalah famili Asteraceae dengan 29 spesies diikuti oleh Leguminosae (19 spesies), Convolvulaceae (9 spesies), Euphorbiaceae (7 spesies) & Amaranthaceae (7 spesies). 57,66% spesies asing yang dilaporkan dari Mizoram berasal dari Amerika diikuti oleh 11,65% dari Afrika, 7,36% dari Meksiko & 5,52% dari Australia. Analisis bentuk kehidupan menunjukkan keberadaan 58,64% tumbuhan, 15,43% pohon dan 9,87% semak. Dari seluruh tumbuhan asing yang tercatat, 91 jenis dimanfaatkan untuk pengobatan tradisional, 43 jenis*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.135-154 

*sebagai tanaman hias, 15 jenis untuk dimakan, sembilan jenis digunakan sebagai kayu dan empat jenis digunakan sebagai pupuk hijau. Studi tersebut juga merekomendasikan sepuluh spesies paling membahayakan, lima tanaman neo-invasif, yang memiliki potensi ancaman terbesar bagi flora asli. Proses dan kemungkinan penyebab invasi di negara bagian juga dibahas secara singkat, yang dapat digunakan dalam penyusunan kebijakan konservasi atau pengelolaan hutan.*

*Kata kunci: Tumbuhan asing, invasif, neo-invasif, keanekaragaman hayati, Mizoram*

## I. INTRODUCTION

Alien plants, introduced or spread outside their natural habitats, have affected natural biodiversity in almost every ecosystem type on earth and are one of the greatest threats to biodiversity (UN, 2014). These plants not only affect the species composition, spatial distribution of the native flora but also impact directly or indirectly on the resources, structures and functions of natural ecosystems (Downey & Richardson, 2016). Efficient mechanism of seed dispersal, high growth rate, great adaptability to wide ranges of environmental conditions are some of the essential processes for the successful establishment of alien plants (Simberloff, Parker, & Windle, 2005). Species that can withstand a wide range of environmental conditions show a broader physiological niche and are likely to be more invasive (Higgins & Richardson, 2014). Almost 0.5–0.7% of global woody plant species is currently invasive outside their natural range (Rejmánek & Richardson, 2013).

Invasive alien species can be recognized as a critical component of global environmental change because, after establishment, they randomly proliferate in all direction; mainly when the environment is conducive as in a rough and sloppy mountain terrains (Rumlerová, Vilà, Pergl, Nentwig, & Pyšek, 2016). The recent global climate change has also catalysed the rate of introduction and spread of alien species into areas where they were previously absent, or increased their performance as compared to native species. Evidence is quite common when many invasive alien species viz. *Ageratum conyzoides*, *Ageratina adenophora*, *Chromolaena odorata*, *Lantana camara*, *Mikania micrantha* and *Parthenium hysterophorus* have brought about havoc in terrestrial ecosystems, while *Eichhornia crassipes*, *Pistia stratiotes* in aquatic ecosystems by significantly changing the structure and composition of native vegetation (Raizada, Sharma, & Raghubanshi, 2008).

Taxonomic identity of alien species, their origin, distribution, spread pattern, habitat and mode of introduction are the prerequisite for any management strategy against invasive species. The entire region of Northeast India including Mizoram, Khasia and Jaintia hills (K&J hill), Lushai Hills, Manipur were under Assam province during the colonial era. *Mikania micrantha* was introduced during World War-II as a ground cover for tea plantations and camouflaged the airfields in this region (Shankar, Yadav, Rai, & Tripathi, 2011). Therefore, the introduction of the alien plants in Mizoram might have followed the path K&J hill- Silchar-Aizawl-Lushai hills and subsequently spread throughout the state. Report on the collection of *Mikania micrantha* dated back to 1956, *Chromolaena odorata* in 1938, *Ageratina riparia* in 1958 and *Ageratina adenophora* in 1931 from this region.

Sporadic information is available on alien species, particularly on Indian Himalayan regions. Ecological studies on invasive plants, mainly on *Ageratum conyzoides*, *Parthenium hysterophorus* and *Lantana camara* were reported from the north-western Himalayan region (Kohli, Batish, Singh, & Dogra, 2006); 571 alien species enumerated from the Kashmir Himalayas (Khuroo, Reshi, Rashid, & Dar, 2011); 190 alien species identified from Indian Himalayan region (Sekar, Manikandan, & Srivastava, 2012); 497 alien species enumerated

from Himachal Pradesh (Jaryan, Uniyal, Gupta, & Singh, 2013). However, accurate estimation on the spread of invasive species and its potential threats is not available from North-eastern states of India, particularly from Mizoram. A proper estimation of floristic elements or landscapes infested with invasive alien plants is the need of the hour to build appropriate strategies for conservation and management of natural flora. Keeping in mind these problems, the present study was carried out in the state of Mizoram (a) to prepare a comprehensive inventory of alien plant species in Mizoram and (b) to evaluate the invasion status of the ten most obnoxious invasive alien plant species; and to propose the five neo- invasive alien plant species, which possess potential threats to the local flora.

## II. MATERIAL AND METHOD

### A. Study Site

The state of Mizoram is one of the smallest north-eastern states of India situated in the extreme eastern part of India (Figure 1); the state is bounded by two international boundaries viz., Myanmar (Burma) on the east and Bangladesh on the west; Tripura, Assam and Manipur also share a common border with the state. The forest cover of the state is 73.68% of the state's total geographical area out of which only 6.75% is under protected area network. Summer temperature varies from 18°C to 29°C and winter temperature varies from 11°C to 24°C. The average annual rainfall of the state ranges between 2160 mm to 3500 mm (FSI, 2019). For the convenience of the present work, we have selected four protected

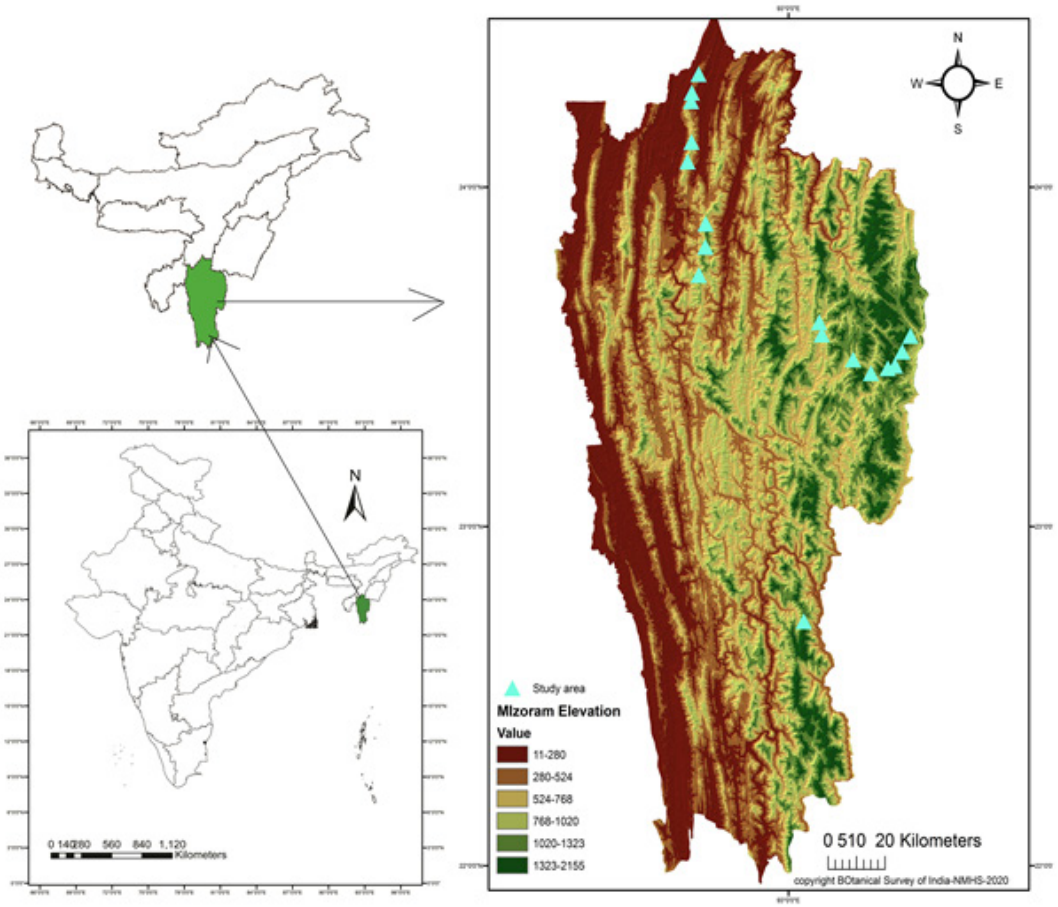


Figure 1. Map of the study site

areas viz. Phawngpui National Park (50 $km^2$), Murlen National Park (100 $km^2$), Lengteng Wildlife Sanctuary (60 $km^2$) and Dampa Tiger Reserve (500 $km^2$). Besides non-protected areas viz. Sangau, Aizawl, Knahlan were also selected. The vegetation of the state can be broadly categorized in to tropical (up to 900 m), subtropical (900−1800 m) and temperate (1800–3600 m) depending on the elevation and precipitation. All the studied plots were situated in subtropical and temperate regions of the state.

**B. Methods**

The present study was carried out during July 2018 to September 2019 as a part of the collaborative research project "Ecological Investigations to understand causes and consequences of invasion in Tripura & Mizoram" under the National Mission on Himalayan Studies (NMHS). Intensive field surveys were undertaken in a planned manner in different seasons to collect the plant specimens from selected protected and unprotected areas of Mizoram. Plant samples were collected for preparation of voucher herbarium specimens. The specimens were poisoned with 0.1% Mercuric Chloride ($MgCl_2$), mounted on standard handmade herbarium sheets (28 × 42 cm) and labelled (14.5 × 11 cm), after incorporating all the relevant field information. These were deposited in Central National Herbarium, Botanical Survey of India (CAL) after proper identification. An exhaustive inventory of alien plants of the state was prepared based on the collections from the field and also by examining the herbarium specimens from multiple herbaria (CAL, ASSAM and ARUN) of the region. Based on the mode of introduction, rate of invasion, nature of the invaded habitats and importance value index (IVI) ten most obnoxious invasive alien plants and five top neo-invasive species were grouped. For the collection of data on the mode of introduction and usefulness of alien plants, the local people were interviewed with an open-ended questionnaire.

Random sampling technique using nested quadrat method was followed for collection of phytosociological data. Plot sizes of 400 $m^2$ were selected randomly within which quadrats of 5 m × 5 m were laid for shrubs and 1 m × 1 m for herbs nested within 5 m × 5 m quadrats (Misra, 1968). All the invasive species occur within each plot were counted along with their associated species. Trees were not considered in the present study because their invasion status is negligible compared to the shrub and herb invasion in the study areas. For the collection of data on the mode of introduction and usefulness of alien plants, the local people were interviewed with an open-ended questionnaire.

**C. Data Analysis**

All the phytosociological data i.e., relative densities, relative frequency, relative dominance, IVI of each species were determined using formulas mentioned by (Misra, 1968). Importance Value Index (IVI) was calculated by the addition of relative values of frequency, density and dominance (Curtis & McIntosh, 1950).

The formulae used for the various calculations are:

$$\text{Density} = \frac{\text{No. of individuals of a species}}{\text{Total No. of quadrats studied}} \quad \text{.....(1)}$$

$$\text{Frequency (\%)} = \frac{\text{No. of quadrats of occurrence of a species}}{\text{Total number of quadrats studied}} \times 100 \quad \text{.(2)}$$

$$\text{Abundance} = \frac{\text{Total No. of individuals of a species}}{\text{Number of quadrats of occurrence}} \quad \text{.....(3)}$$

$$\text{Relative Frequency} = \frac{\text{Frequency of a species}}{\text{Frequency of all the species}} \times 100 \quad \text{.....(4)}$$

$$\text{Relative Density} = \frac{\text{Density of a species}}{\text{Density of all the species}} \times 100 \quad \text{.....(5)}$$

$$\text{Relative Dominance} = \frac{\text{Basalarea of a species}}{\text{Basal area of all species}} \times 100 \quad \text{.....(6)}$$

Where: Basal area = $\pi r^2$, $\pi$ = 3.14 and r = radius of the species; Importance Value Index (IVI) = Relative Frequency + Relative Density + Relative Dominance

Importance Value Index (IVI) was calculated separately for each species of the community. A particular species, that having highest value of importance value index (IVI) is considered as most dominant in the area and the species with lowest importance value is considered as the least dominant.

The comparisons among the phytosociological data were analysed in Microsoft Excel Software (2019 version). GPS locations were investigated and the digital elevation map of the study site was prepared using ArcGIS online tools. Based on the mode of introduction, rate of invasion, nature of the invaded habitats and importance value index (IVI) ten most obnoxious alien invasive alien plants and five top neo-invasive species were determined.

## III. RESULT AND DISCUSSION

Extensive field exploration tours undertaken in the protected and non-protected areas of Mizoram resulted in a collection of more than 1045 plant samples. During the study, 163 alien plant species were recorded from Mizoram belonging to 135 genera and 51 families (See Appendix 1, Table 1). Out of these, 147 species belongs to 122 genera and 43 families were dicotyledons; 11 species belongs to 9 genera and five families were monocotyledons. Five species of gymnosperms under four genera and three families were also recorded.

Table 2. Top ten families among the alien plants (with minimum fourspecies)

| No. | Family | Species |
|---|---|---|
| 1. | Asteraceae | 29 |
| 2. | Leguminosae (s.l.) | 19 |
| 3. | Convolvulaceae | 9 |
| 4. | Euphorbiaceae | 7 |
| 5. | Amaranthaceae | 7 |
| 6. | Malvaceae | 6 |
| 7. | Solanaceae | 7 |
| 8. | Verbenaceae | 5 |
| 9. | Acanthaceae | 4 |
| 10. | Poaceae | 4 |

Besides, out of the total reported plant species, 95 plant species were represented by herbs (58.28%), 25 trees (15.33%), 20 shrubs (12.26%), 14 climbers (8.58%), four grasses (2.45%) and only two species of sedges (1.27%). Maximum percentage of alien plants, i.e., 38.03% (62 taxa) occur in Mizoram were from Tropical American origin followed by 13.49% (22 taxa) from South American, 9.81% (16 taxa) from Tropical African, 7.3% (12 taxa) from Mexican and 5.5% (9 taxa) from Australian origin. The plants belonging to the different nativity was given in Figure 2.

The most dominant families with maximum species diversity shown in Table 2. Asteraceae with 29 species represented was the most dominant family followed by Leguminosae, Convolvulaceae, Euphorbiaceae,

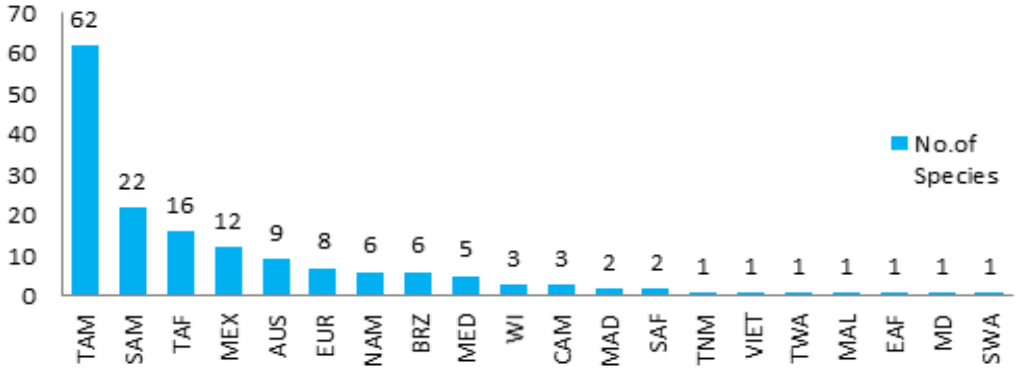


Figure 2. Nativity of the different invasive alien species found in Mizoram flora

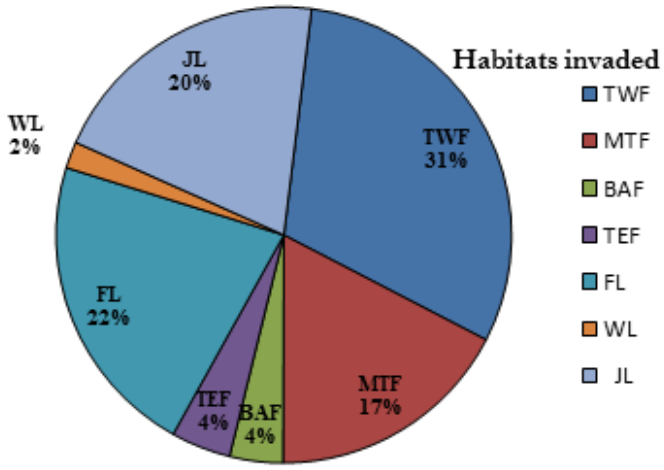


Figure 3. Habitats invaded by the invasive alien plant

Amaranthaceae, and Malvaceae. Ten most dominating families contribute 59.06% of total alien species found.

Among the most dominant families, two families viz., Asteraceae, Convolvulaceae were also reported as dominant in Indian Himalayan region (Sekar, Manikandan, & Srivastava, 2012) as well as throughout India (Reddy, 2008). The dominance of these two families as effective invaders in natural forests may be attributed to the high production of viable seeds which usually dispersed by wind in Asteraceae, and successful vegetative reproduction in members of Convolvulaceae.

Observation on invaded habitats revealed that Tropical Wet Evergreen forest (31%) and Montane sub-tropical forest (17%) were most invaded forests whereas Fallow Lands (22%) and Jhumlands (20%) were invaded habitats outside the forest area (Figure 3). It is also found that 52.5% of alien plants were perennials and 47.5% annuals. The percentage of perennial alien plant contribution in Mizoram (52.5%) is higher than the average contribution of Indian Himalayan region (37%) implies that alien species are probably well established in Mizoram, or natural vegetation is more vulnerable. A moderate-higher percentage of annual alien species in the state indicates, the forest is degraded and also substantiate our findings of the gregarious spread of invasive species in mountain slopes (Figure 6). The preferred habitat of spreading of invasive species in different habitat in Mizoram is almost similar to other Indian Himalayan region (Sekar, Manikandan, & Srivastava, 2012) as well as throughout India (Reddy, 2008). The most preferred habitats of roadsides, wetlands and fallow lands may be attributed to low competition and high disturbances in these regions.

An open-ended interview with the local villagers, forest officials and in-depth observation on the probable mode of introduction of the alien species revealed, 62.34% of the total alien species possibly introduced unintentionally while 29.62% of species were introduced for ornamental purpose and 8.02% were introduced as a food (Figure 4).

The biodiversity of a forest area controls the economy of the surrounding villages and partially fulfils their basic requirements like food, medicine, thatching material, and fodder etc. Study on the use pattern of the alien species among the local inhabitants revealed that 91 species had been used as medicinal purposes, 43 species as ornamental, 15 species as foods, nine species as fuel woods, four species as green manure and use of 6 species were not known to the locals (Figure 5). *Acmella oleracea* (flowers, stems), *Solanum torvum* (seeds) were

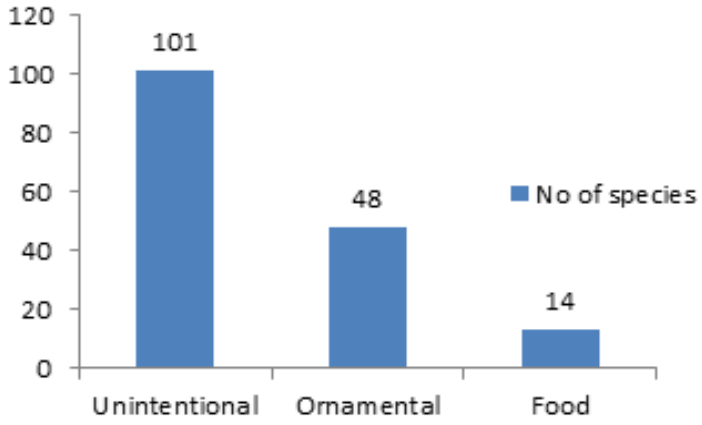


Figure 4. Mode of the introduction of the invasive alien plants in Mizoram

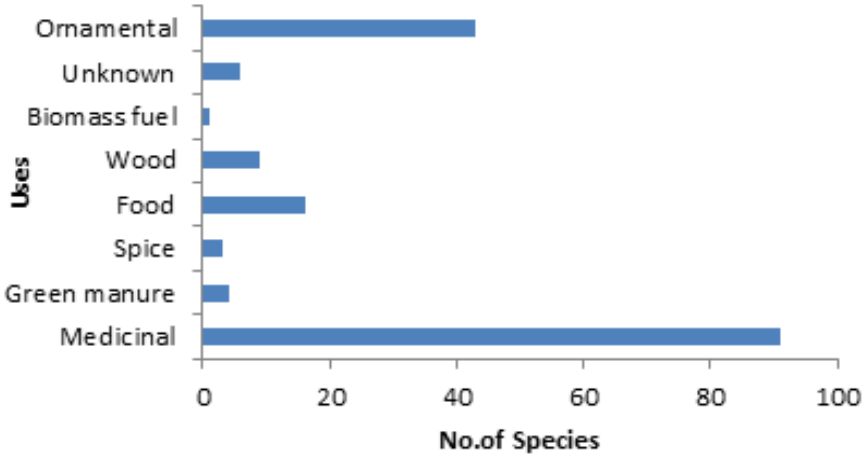


Figure 5. Uses of alien plants in the state

used for curing toothache; *Bidens pilosa* (leaf juice) was used for swollen glands and as eye-drops. Crushed leaf juice of *Chromolaena odorata*, *Mikania micrantha* were applied externally on cuts and wounds as antiseptic; leaves of *Oxalis corniculata* were eaten raw to reduce stomach ache. Leaf juice of *Achyranthus aspera* and *Ageratum conyzoides* were applied on sore-legs of domestic animals. Dried inflorescences of *Ocimum americanum* and dried leaves *Eryngium foetidum* were used as condiments in local cuisine. Tender fruit pods of *Leucaena leucocephala* were boiled with other vegetables for food. *Argemone mexicana* extract was used for adulteration purpose with mustard oil. *Annona reticulata* and *Annona squamosa* fruits were consumed widely by the locals. Leaves of *Saccharum spontaneum* and *Typha angustifolia* were used for rope making and thatching purpose. Brooms made up of dried leafless stems of *Sida acuta* were used for household cleaning purposes.

Based on the phytosociological data, ten most invasive alien plants in four protected areas (Phawngpui National Park, Murlen National Park, Lengteng Wildlife Sanctuary, Dampa Tiger reserve) were observed highly proliferative. In Phawngpui national park, *Ageratina adenophora* (IVI-62.76), *Hypoestis phyllostachya* (IVI-29.06), *Ageratina riparia* (IVI-50.91) were the most dominant invasive alien species with associated native species like *Lobelia pyramidalis* (IVI-54.46), *Lindenbergia grandiflora* (IVI-52.27), *Anisochilus carnosus* (IVI-37.55) and *Ainsliaea latifolia* (IVI-36.01) occurring between

1400-2250 m of elevation whereas *Chromolaena odorata* (IVI-45.83), *Mikania micrantha* (IVI-59.64), *Imperata cylindrica* (IVI-24.83) were observed dominant over native species like *Strobilanthes maculata* (IVI-21.20) and *Impatiens stenantha* (IVI-32.53) up to 1550 m of altitude only. In Phawngpui National Park, frequency of native species was higher compared to other three protected areas. In Murlen National Park, native plants like *Ainsliaea latifolia* (IVI-23.65), *Oplismenus burmanii* (IVI-20.04) and *Strobilanthes maculata* (IVI-18.09) associated with dominant invasive plants *Ageratina adenophora* (IVI-57.94), *Ageratina riparia* (IVI- 46.11), *Mikania micrantha* (IVI-57.10) and *Lantana camara* (IVI-44.48), *Chromolaena odorata* (IVI-37.05) were observed in 700-2149 m of elevation. In Lengteng Wildlife Sanctuary, between 400–1250 m of altitude, *Impatiens stenantha* (IVI-20.16), *Eragrostis curvula* (IVI-20.74), *Oplismenus burmanii* (IVI-23.96), Strobilanthes maculata (IVI-17.75) and *Osbeckia chinensis* (IVI-28.88) were dominant associated native species with invasive alien plants like *Chromolaena odorata* (IVI-35.76), *Lantana camara* (IVI-39.3). Whereas in between 1250–2141 m of elevation, the spread of *Ageratina adenophora* (IVI-55.31), *Ageratina riparia* (IVI-77.23), *Mikania micrantha* (IVI-50.42) were most abundant suppressing associated native species like *Lobelia pyramidalis* (IVI- 11.11), *Lindenbergia grandiflora* (IVI-13.33), *Anisochilus carnosus* (IVI-22.96) and *Ainsliaea latifolia* (IVI- 8.89). In Dampa Tiger Reserve, between 800–1100 m of elevation, *Mikania micrantha* (IVI-45.39), *Lantana camara* (IVI-47.34), *Ageratum houstonianum* (IVI-45.33), *Bidens pilosa* (IVI-39.15), *Ageratum conyzoides* (IVI-66.86) were observed as most abundant invasive species showing dominance over native species like *Osbeckia chinensis* (IVI-31.89), *Elatostema sessile* (IVI-31.90), *Impatiens stenantha* (IVI-22.44) (Table 3). It has also been observed that *Ageratina adenophora*, *Ageratina riparia*, *Chromolaena odorata* spreading throughout Mizoram between 700 m to 2200 m of elevation.

The gregarious spread of these invasive species greatly hinders regeneration of secondary forests and also crops probably due to their allelopathic effect and growth inhibitors. A similar result was also reported from agricultural lands of eastern Himalayas (Shankar, Yadav, Rai, & Tripathi, 2011). The other significant impacts of alien plants on native biodiversity are usually changed soil nutrient composition and species richness. The main reasons for the great invasion in these areas may be attributed to the construction of roads, laying of water pipelines and electric poles through forest areas and forest clearance for agricultural lands. A similar observation was also observed in Siwalik low land forests of western Himalaya where 50–64 % density of native flora were lost due to invasion of *Ageratum conyzoides* (Batish, Kaur, Singh, & Kohli, 2009). Reduction in productivity and increase of maintenance cost was caused by fast-spreading of invasive alien plants in agriculture ecosystems (Dogra, Kohli, Sood, & Dobhal, 2009).

Besides the ten most obnoxious invasive species, other new potential invasive plants observed in Phawngpui national park and Lengteng wildlife sanctuary were *Tithonia diversifolia* (IVI-33.41, 26.24), *Ipomoea hederifolia* (IVI-35.80, 36.50), *Ipomoea cairica* (IVI-29.10, 25.18), *Crassocephalum crepidioides* (IVI-45.53, 11.51), *Galinsoga parviflora* (IVI-21.12, 20.30). We have observed only one potential new invasive species *Ipomoea hederifolia* (IVI-37.53) in Murlen national park and two potential new invasive species *Ipomoea cairica* (IVI-46.58), *Crassocephalum crepidioides* (IVI-22.62) in Dampa tiger reserve in Mizoram. These new invasive species were firstly invading into the natural vegetation regions and posed a significant threat to it (Table 4).

From the present study, it can be concluded that *Ageratina adenophora*, *Ageratina riparia*, *Mikania micrantha*, *Lantana camara*, *Ageratum houstonianum*, *Chromolaena odorata*, *Hypoestis phyllostachya*, *Bidens pilosa*, *Imperata cylindrica*, *Ageratum conyzoides* are the most harmful alien plants responsible for a high-volume invasion in forest lands, roadsides and fallow lands which causing noticeable damage to the flora of Mizoram. Besides five most neo-invasive species, namely *Tithonia diversifolia*, *Ipomoea hederifolia*, *Ipomoea*

Table 3. Phytosociological information of ten most-noxious invasive alien species in protected areas in Mizoram

| Name of the plants | Frequency | | | | Density | | | | Abundance | | | | IVI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR |
| *Ageratina adenophora* | 90 | 80 | 70 | - | 12.5 | 17.5 | 14.9 | - | 13.88 | 21.87 | 21.28 | - | 62.76 | 57.94 | 55.31 | - |
| *Ageratina riparia* | 70 | 70 | 80 | - | 10.80 | 14.9 | 17.5 | - | 15.43 | 21.29 | 21.88 | - | 50.91 | 46.11 | 77.23 | - |
| *Mikania micrantha* | 100 | 75 | 100 | 40 | 30.29 | 13.13 | 24.14 | 10.8 | 30.29 | 17.50 | 24.14 | 27 | 59.64 | 57.10 | 50.42 | 45.39 |
| *Hypoestis phyllostachya* | 60 | 10 | 40 | - | 8.1 | 0.3 | 2.6 | - | 13.5 | 3.00 | 6.50 | - | 29.06 | 8.52 | 21.54 | - |
| *Lantana camara* | - | 50 | 28.57 | 40 | - | 4.75 | 5.86 | 13 | - | 9.50 | 20.50 | 32.5 | - | 44.48 | 35.76 | 47.34 |
| *Ageratum houstonianum* | 70 | - | 40 | 50 | 9.50 | - | 3.8 | 9.5 | 13.57 | - | 9.50 | 19 | 35.96 | - | 24.2 | 45.33 |
| *Chromolaena odorata* | 28.57 | 70 | 57.14 | 60 | 5.86 | 5.5 | 5.57 | 4.8 | 20.50 | 7.85 | 9.75 | 8.00 | 45.83 | 37.05 | 39.35 | 45.26 |
| *Bidens pilosa* | 50 | 30 | 30 | 80 | 3.2 | 1.5 | 3.1 | 16.5 | 6.40 | 5.00 | 10.33 | 20.62 | 20.74 | 22.52 | 28.57 | 39.15 |
| *Imperata cylindrica* | 50 | 30 | 30 | - | 2.8 | 2.7 | 2.7 | - | 20.00 | 9.00 | 9.00 | - | 24.83 | 19.74 | 23.58 | - |
| *Ageratum conyzoides* | 20 | 60 | 20 | 90 | 0.60 | 7.3 | 2.5 | 23.1 | 3.00 | 12.16 | 12.50 | 25.66 | 10.63 | 18.88 | 25.35 | 66.86 |

Remarks: PNP=Phawngpui National Park, MNP= Murlen National Park, LWS= Lengteng wildlife Sanctuary, DTR=Dampa Tiger Reserve

Table 4. Phytosociological information of top ten dominant native species in protected areas in Mizoram

| Name of the plants | Frequency (%) | | | | Density | | | | Abundance | | | | IVI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR | PNP | MNP | LWS | DTR |
| *Lobelia pyramidalis* | 60 | – | 50 | – | 4.2 | – | 3.20 | – | 7.00 | – | 6.40 | | 54.46 | – | 11.11 | – |
| *Lindenbergia grandiflora* | 30 | – | 60 | 40.00 | 1.7 | – | 3.40 | 2.70 | 5.67 | – | 5.67 | 6.75 | 52.27 | – | 13.33 | 28.55 |
| *Anisochilus carnosus* | 20 | – | 30 | – | 2.8 | – | 2.10 | | 14 | – | 7.00 | | 37.55 | – | 22.96 | |
| *Ainsliaea latifolia* | 40 | 30 | 40 | 3.8 | – | 3.1 | 3.20 | – | 9.50 | 10.33 | 8.00 | – | 36.01 | 23.65 | 8.89 | – |
| *Impatiens stenantha* | 30 | – | 20 | 20.00 | 2.5 | 1.30 | 1.60 | 1.70 | 8.33 | 6.50 | 8.00 | 8.50 | 32.53 | – | 20.16 | 22.44 |
| *Eragrostis curvula* | 60 | 30 | 20 | 40 | 2.5 | 2.7 | 1.40 | 2.80 | 17.86 | 9.00 | 7.00 | 7.0 | 28.74 | 19.74 | 20.74 | 27.17 |
| *Oplismenus burmanii* | 60 | 20 | 30 | – | 1.5 | 2.2 | 1.80 | – | 15.00 | 11.00 | 6.00 | – | 27.41 | 20.04 | 23.96 | – |
| *Strobilanthes maculata* | 50 | 40 | 20 | – | 2.2 | 2.6 | 1.50 | – | 12.22 | 6.50 | 7.50 | – | 21.20 | 18.09 | 17.75 | – |
| *Osbeckia chinensis* | – | – | 20 | 30.00 | – | – | 2.80 | 1.90 | – | – | 14.00 | 6.33 | – | – | 28.88 | 31.89 |
| *Elatostema sessile* | – | – | 40 | 50.00 | – | – | 0.90 | 3.70 | – | – | 2.25 | 7.40 | – | – | 17.75 | 31.90 |

Remarks: PNP=Phawngpui National Park, MNP= Murlen National Park, LWS= Lengteng wildlife Sanctuary, DTR=Dampa Tiger Reserve

ISSN: 2355-7079/E-ISSN: 2406-8195

Table 5. Phytosociological data of five neo-invasive alien species in protected areas in Mizoram

| Name of the plants | Frequency | | | | Density | | | | Abundance | | | | IVI | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PNP | MNP | LWS | DTS | PNP | MNP | LWS | DTS | PNP | MNP | LWS | DTS | PNP | MNP | LWS | DTS |
| *Tithonia diversifolia* | 57.14 | - | 42.86 | - | 6.71 | - | 5.71 | - | 11.75 | - | 13.33 | - | 33.41 | - | 26.24 | - |
| *Ipomoea hederifolia* | 71.43 | 37.5 | 28.57 | - | 12.43 | 5.00 | 9.14 | - | 17.40 | 13.33 | 32.00 | - | 35.80 | 37.53 | 36.50 | - |
| *Ipomoea cairica* | 42.86 | - | 71.43 | 60 | 4.86 | - | 3.43 | 16.2 | 11.33 | - | 4.80 | 27 | 29.10 | - | 25.18 | 46.58 |
| *Crassocephalum crepidioides* | 60 | - | 20 | 50 | 7.30 | - | 1.6 | 3.5 | 12.17 | - | 8.00 | 7 | 45.53 | - | 11.51 | 22.62 |
| *Galinsoga parviflora* | 50 | 60 | 30 | - | 3.70 | 7 | 1.5 | - | 7.40 | 11.66 | 5.00 | - | 21.12 | - | 20.30 | - |

Remarks: PNP=Phawngpui National Park, MNP= Murlen National Park, LWS= Lengteng wildlife Sanctuary, DTR=Dampa Tiger Reserve

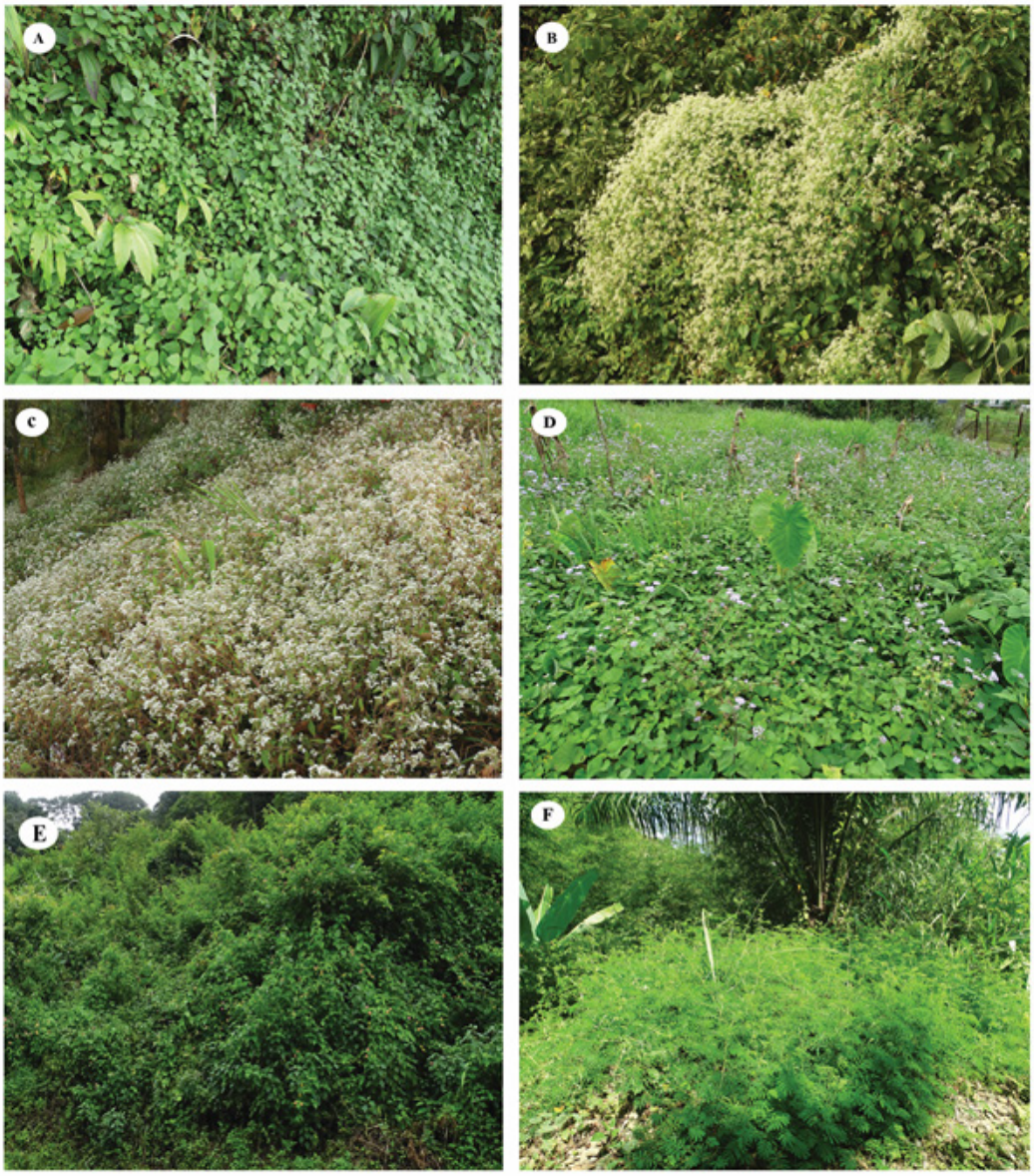


Figure 6. The invasive spread of (A) *Ageratina adenophora* (Spreng.) M.King & H.Rob. (B) *Mikania micrantha* Kunth. (C) *Ageratina riparia* (Regel) R.M.King & H.Rob. (D) *Ageratum houstonianum* Mill. (E) *Lantana camara* L. (F) *Mimosa diplotricha* Wright. in Mizoram, India

*cairica*, *Crassocephalum crepidioides*, *Galinsoga parviflora* are the most potential invasive species spreading aggressively between 500 m to 1800 m of elevation (Table 5). The most probable reasons for these rapid invasions and declining biodiversity may be attributed to allelopathic effect, fragile soil, a high influx of tourist, land clearance and the livestock-dependent lifestyle of local inhabitants of this region (Chen, Peng, & Ni, 2009).

Some of the successful strategies which has been used to control invasive species include: (i) ecological restoration by allowing selected indigenous plant species with potential to outcompete invasive species to flourish, (ii) mechanical control in combination with crop-competition method, and (iii) cut-root-stock method combined with introduction of native legumes and grasses (CBD, 2019), chemical control and biological control (Singh, 2017).

*Lantana camara* was reported to be efficient in heavy metal as well as particulate pollution phytoremediation in Mizoram which shows promising use of invasive plants in pollution abatement phytotechnologies to assist in their sustainable management (Rai & Singh, 2015). Education and awareness programmes developed by different agencies were essential which helped to restrict the spread of invasive species in the state. In this connection, during the study period, two awareness programmes were organized by the authors in collaboration with state Forest Department in Phawngpui national park and Mizoram University which had a great positive impact in the local stakeholders. Department of Environment, Forests and Climate Change, Government of Mizoram has taken up the task of developing a suitable framework to Control and Eradication of Forest Invasive Species in Mizoram (Singh et al., 2016).

## IV. CONCLUSION

Mizoram is a biodiversity-rich state and invasion of alien species is emerging as a significant concern for the conservation of native flora. Majority of the population are forest dwellers and mainly dependant on forest resources. Therefore, first-hand information on native flora and invasive flora is needed for formulating scientific management strategy for long-term sustainable utilization. Controlled practices of traditional slash-and-burn cultivation, strict quarantine measures for importing of food crops and introduction of exotic plants are needed to be streamlined to strengthen a strategy for proper utilization, generate livelihood and wellbeing of the society. Regular awareness programmes may also to be conducted to appraise the local inhabitants about the impacts and control of invasive alien plants.

## ACKNOWLEDGEMENT

The authors are grateful to Dr A.A. Mao, Director, Botanical Survey of India, Kolkata for encouragement and facility. The authors express their gratitude to the Department of Environment, Forest and Climate Change, Government of Mizoram for granting permission to survey and conduct this study. The authors are grateful to the Ministry of Environment, Forest and Climate Change, Government of India for financial support through the National Mission on Himalaya Studies (Project NMHS-2017/LG/01/475).

The authors express no conflict of interest.

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Appendix 1.

Table 1. List of Alien Plants in Mizoram and their range of distribution:

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 1. | *Acacia auriculiformis* Benth. | Leguminosae(s.l.)[1] | D | AUS | P | T | OR | TWF | Med |
| 2. | *Acmella radicans* (Jac.) R. R. Janses | Asteraceae | D | MEX | P | H | U | JL | Med |
| 3. | *Aeschynomene americana* L. | Leguminosae(s.l.)[3] | D | TAM | P | H | U | MTF | Man |
| 4. | *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. | Asteraceae | D | MEX | P | H | OR | MTF, TEF | Man |
| 5 | *Ageratina riparia* (Regel) R.M.King & H. Rob. | Asteraceae | D | CAM | P | H | U | TEF | Man |
| 6. | *Ageratum houstonianum* Mill. | Asteraceae | D | TAM | A | H | OR | TWF, MTF | Bio |
| 7. | *Ageratum conyzoides* (L.) L. | Asteraceae | D | TAM | A | H | OR | TWF, MTF | Med |
| 8. | *Alternanthera paronychioides* St. Hill. | Amaranthaceae | D | TAM | A | H | U | TWF | Med |
| 9. | *Alternanthera philoxeroides* (Mahrt.) Griseb. | Amaranthaceae | D | TAM | A | H | F | TWF | Food |
| 10. | *Alternanthera sessilis* (L.) DC. | Amaranthaceae | D | TAM | A | H | U | TWF | Med |
| 11. | *Alternanthera ficoidea* (L.) Sm. | Amaranthaceae | D | TAM | A | H | U | TWF | Med |
| 12. | *Althea rosea* (L.) Cav. | Malvaceae | D | EUR | P | H | OR | JL | Orn |
| 13. | *Amaranthus spinosus* L. | Amaranthaceae | D | TAM | A | H | F | JL | Food |
| 14. | *Ammannia baccifera* L. | Lythraceae | D | TAM | A | H | U | JL | Med |
| 15. | *Anagallis arvensis* L. | Primulaceae | D | EUR | A | H | U | JL | Med |
| 16. | *Anethum graveolens* L. | Apiaceae | D | SWA | A | H | F | JL | Sp |
| 17. | *Annona reticulata* L. | Annonaceae | D | TAM | P | T | F | JL | Food |
| 18. | *Annona squamosa* L. | Annonaceae | D | TAM | P | T | F | JL | Food |
| 19. | *Antigonon leptopus* Hook. & Arn. | Polygonaceae | D | TAM | P | CL | U | JL | Orn,Med |
| 20. | *Apium graveolens* L. | Apiaceae | D | EUR | A | H | F | JL | Sp,Med |
| 21. | *Araucaria columnaris* (G.Forst.) Hook. | Araucariaceae | G | AUS | P | T | OR | TEF | T |
| 22. | *Araucaria excelsa* F. Br | Araucariaceae | G | AUS | P | T | OR | TEF | T |
| 23. | *Argemone mexicana* L. | Papaveraceae | D | SAM | A | H | U | JL | Ad,Med |
| 24. | *Artemisia vulgaris* L. | Asteraceae | D | EUR | P | H | U | MTF | Med |
| 25. | *Asclepias curassavica* L. | Asclepiadaceae | D | TAM | A | H | OR | MTF | Med |
| 26. | *Bidens pilosa* L. | Asteraceae | D | TAM | A | H | U | MTF,JL, FL | Med |
| 27. | *Bixa orrellana* L. | Bixaceae | D | BRZ | P | T | U | JL | Med |

Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 28. | *Blainvillea acmella* (L.) Philipson | Asteraceae | D | TAM | A | H | U | JL,TWF | Med |
| 29. | *Blumea lacera* (Burm.f.) DC. | Asteraceae | D | TAM | A | H | U | TWF | Med |
| 30. | *Bougainvillea glabra* Choisy | Nyctaginaceae | D | BRZ | P | L | OR | TWF | Orn |
| 31. | *Bougainvillea spectabilis* Willd. | Nyctaginaceae | D | BRZ | P | L | OR | TWF | Orn |
| 32. | *Brassica oleracea* L. | Brassicaceae | D | EUR | P | H | F | JL | Food |
| 33. | *Brunfelsia americana* L. | Solanaceae | D | WI | P | SH | OR | JL | Orn |
| 34. | *Brunfelsia hopeana* Benth. | Solanaceae | D | TAM | P | SH | U | JL | Orn |
| 35. | *Caesalpinia bonduc* (L.) Roxb. | Leguminosae(s.l.)[2] | D | TAF | P | T | U | TWF | Med |
| 36. | *Caladium bicolor* (Aiton) Vent. | Araceae | M | SAM | P | H | OR | TWF | Orn |
| 37. | *Calliandra haematocephala* Hassk. | Leguminosae(s.l.)[1] | D | TAM | P | SH | OR | TWF | Orn |
| 38. | *Callistemon citrinus* (Curtis) Skeels | Myrtaceae | D | AUS | P | T | OR | TWF | Med |
| 39. | *Calotropis gigantea* (L.) R. Br. | Asclepiadaceae | D | TAF | P | SH | OR | TWF | Med |
| 40. | *Calotropis procera* (Ait.) R. Br. | Asclepiadaceae | D | TAF | P | SH | U | TWF, FL | Med |
| 41. | *Cardamine hirsuta* L. | Brassicaceae | D | TAM | A | H | U | FL | Unk |
| 42. | *Cascabela thevetia* (L.) Lippold | Apocynaceae | D | MEX | P | T | OR | FL | Orn |
| 43. | *Cassia fistula* L. | Leguminosae(s.l.)[1] | D | NAM | P | T | U | FL,TWF | M |
| 44. | *Casuarina equisetifolia* L. | Casuarinaceae | D | AUS | P | T | OR | MTF,TEF | Orn,T |
| 45. | *Catharanthus roseus* (L.) G. Don. | Apocynaceae | D | TAM | P | H | OR | FL | Med |
| 46. | *Cayratia trifolia* (L.) Domin | Vitaceae | D | AUS | P | CL | U | TWF ,FL | Med |
| 47. | *Celosia argentea* L. | Amaranthaceae | M | TAF | A | H | F | MTF,FL | Orn |
| 48. | *Ceratophyllum demersum* L. | Ceratophyllaceae | M | TAM | A | H | U | WL | Orn |
| 49. | *Chloris barbata* Sw. | Poaceae | M | TAM | P | GR | U | MTF ,FL | Fo |
| 50. | *Chromolaena odorata* (L.) R.M. King & H. Rob. | Asteraceae | D | SAM | P | H | U | MTF,BAF,FL | Med |
| 51. | *Cleome rutidosperma* DC. | Capparaceae | D | TAM | A | H | U | TWF | Med |
| 52. | *Cleome viscosa* L. | Capparaceae | D | TAM | A | H | U | TWF | Med |
| 53. | *Clerodendrum chinense* (Osbeck) Mabb. | Verbenaceae | D | VIET | P | SH | U | TWF | Med |
| 54. | *Corchorus aestuans* L. | Tiliaceae | D | SAM | A | H | U | JL, FL | Food |

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Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 55. | *Corymbia maculata* (Hook.) K.D.Hill & L.A.S.Johnson | Myrtaceae | D | AUS | P | T | OR | JL, FL | T |
| 56. | *Cosmos bipinnatus* Cav. | Asteraceae | D | MEX | A | H | OR | JL, FL | Orn |
| 57. | *Crassocephalum crepidioides* (Benth.) S. Moore | Asteraceae | D | TAM | A | H | U | MTF | Med |
| 58. | *Crotalaria pallida* Ait. | Leguminosae(s.l.)[3] | D | TAM | A | H | U | TWF | Med |
| 59. | *Croton bonplandianus* Baill. | Euphorbiaceae | D | SAM | P | H | U | TWF | Fo |
| 60. | *Cupressus sempervirens* L. | Cupressaceae | G | AUS | P | T | U | TEF | T,Med |
| 61. | *Cuscuta chinensis* Lam. | Convolvulaceae | D | MED | P | CL | U | MTF, BAF | Med |
| 62. | *Cuscuta reflexa* Roxb. | Convolvulaceae | D | MED | A | CL | U | MTF, BAF | Med |
| 63. | *Cyanthillium cinereum* (L.) H. Rob. | Asteraceae | D | TAF | A | H | U | TWF | Med |
| 64. | *Cyperus difformis* L. | Cyperaceae | M | TAM | A | SE | U | TWF | Fo |
| 65. | *Cyperus iria* L. | Cyperaceae | M | TAM | A | SE | U | TWF | Fo |
| 66. | *Datura metel* L. | Solanaceae | D | TAM | P | SH | U | TWF | Med |
| 67. | *Datura stramonium* L. | Solanaceae | D | TAM | P | SH | U | TWF | Med |
| 68. | *Delonix regia* (Hook.) Raf. | Leguminosae(s.l.)[2] | D | MAD | P | T | OR | TWF,MTF | Orn |
| 69 | *Dombeya mastersii* Hook. f. | Sterculiaceae | D | TAF | P | SH | OR | TWF ,FL | Orn |
| 70. | *Duranta erecta* L. | Verbenaceae | D | MEX | P | SH | OR | FL | Orn |
| 71. | *Dysphania ambrosioides* (L.) Mosyakin & Clemants | Chenopodiaceae | D | TAM | P | H | U | FL | Med |
| 72. | *Echinochloa colona* (L.) Link | Poaceae | M | SAM | A | GR | U | JL | Fo |
| 73. | *Eclipta prostrata* (L.) L. | Asteraceae | D | TAM | A | H | U | JL | Med |
| 74. | *Eichhornia crassipes* (Mart.) Solms. | Pontederiaceae | M | TAM | P | H | OR | WL | Man,Wwt |
| 75. | *Emilia sonchifolia* (L.) DC. ex DC. | Asteraceae | D | TAM | A | H | U | TWF, MTF | Med |
| 76. | *Erigeron canadensis* L. | Asteraceae | D | SAM | A | H | U | TWF, MTF | Med |
| 77. | *Erigeron karvinskianus* DC. | Asteraceae | D | MEX | A | H | U | TWF, MTF | Med |
| 78. | *Eschscholzia californica* Cham. | Papaveraceae | D | MEX | P | H | U | TWF, MTF | Med |
| 79. | *Eucalyptus globulus* Labill. | Myrtaceae | D | AUS | P | T | OR | MTF | Med |
| 80. | *Euphorbia milii* Des Moul. | Euphorbiaceae | D | MD | P | H | OR | TWF | Orn |

Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 81. | *Euphorbia pulcherrima* Willd. ex Klotzsch. | Euphorbiaceae | D | MEX | P | SH | OR | TWF, FL | Orn |
| 82. | *Euphorbia hirta* L. | Euphorbiaceae | D | TAM | A | H | U | TWF | Med |
| 83. | *Evolvulus nummularius* (L.) L. | Convolvulaceae | D | TAM | P | H | U | TWF | Unk |
| 84. | *Galinsoga parviflora* Cav. | Asteraceae | D | TAM | A | H | U | MTF | Fo |
| 85. | *Glandularia canadensis* (L.) Small | Verbenaceae | D | SAM | P | H | OR | MTF | Orn |
| 86. | *Gompherena serrata* L. | Amaranthaceae | D | TAM | A | H | U | MTF | Med |
| 87. | *Grangea maderaspatana* (L.) Pori | Asteraceae | D | SAM | P | H | U | TWF | Med |
| 88. | *Hygrophila auriculata* (Schumach.) Heine | Acanthaceae | D | TAF | A | H | F | FL | Food,Med |
| 89. | *Hypoestes phyllostachya* Baker | Acanthaceae | D | SAF | A | H | OR | MTF,BAF | Orn |
| 90. | *Hyptis suaveolens* (L.) Poit. | Lamiaceae | D | TAM | A | H | U | MTF, FL | Med |
| 91. | *Imperata cylindrica* (L.) Raeusch. | Poaceae | M | TAM | P | GR | U | FL | Fo |
| 92. | *Indigofera trita* L.f. | Leguminosae(s.l.)[3] | D | TAF | P | SH | U | TEF | Unk |
| 93. | *Ipomoea alba* L. | Convolvulaceae | D | TAM | P | CL | OR | MTF | Med |
| 94. | *Ipomoea cairica* Sweet | Convolvulaceae | D | MED | P | CL | OR | MTF | Orn |
| 95. | *Ipomoea quamoclit* L. | Convolvulaceae | D | TAM | P | CL | OR | MTF | Orn |
| 96. | *Ipomoea carnea* Jacq. | Convolvulaceae | D | TAM | P | SH | U | JL, FL | Orn |
| 97. | *Ipomoea hederifolia* L. | Convolvulaceae | D | TAM | A | CL | U | JL ,FL | Orn |
| 98. | *Ipomoea purpurea* (L.) Roth | Convolvulaceae | D | SAM | P | CL | OR | JL ,FL | Orn |
| 99. | *Jacaranda mimosifolia* D.Don | Bignoniaceae | D | SAM | P | T | OR | TWF | Orn |
| 100. | *Jatropha gossypifolia* L. | Euphorbiaceae | D | TAM | P | H | U | TWF | Med |
| 101. | *Juniperus communis* L. | Juniperaceae | G | NAM | P | T | OR | TEF | T |
| 102. | *Kalanchoe pinnata* (Lam.) Merr. | Crassulaceae | D | MAD | P | H | OR | FL | Orn,Med |
| 103. | *Kigelia pinnata* Jacq. | Bignoniaceae | D | TAF | P | T | OR | TWF | Med |
| 104. | *Lantana camara* L. | Verbenaceae | D | TAM | P | H | OR | TWF,MTF | Orn,Bas |
| 105. | *Leonotis nepetifolia* (L.) R.Br. | Lamiaceae | D | TAF | A | H | U | TWF | Med |

ISSN: 2355-7079/E-ISSN: 2406-8195

Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 106. | *Leucaena leucocephala* (Lam.) de Wit | Leguminosae(s.l.)[1] | D | MEX | P | T | U | TWF | Food |
| 107. | *Ludwigia adscendens* (L.) Hara. | Onagraceae | D | TAF | A | H | U | FL | Med |
| 108. | *Ludwigia perennis* L. | Onagraceae | D | TAF | A | H | U | FL | Food,Med |
| 109. | *Magnolia grandiflora* L. | Magnoliaceae | D | NAM | P | T | U | MTF | Orn |
| 110. | *Malvastrum coromandelianum* (L.) Garcke | Malvaceae | D | TAM | A | H | U | TWF, BAF | Orn |
| 111. | *Malvaviscus palmanus* Pittier & Donn. Sm. | Malvaceae | D | TAM | P | H | OR | BAF | Orn |
| 112. | *Manihot esculenta* Crantz. | Euphorbiaceae | D | TAM | P | T | U | MTF | Food,Med |
| 113. | *Mecardonia procumbens* (Mill.)Small | Scrophulariaceae | D | TNM | A | H | U | JL,FL,MTF | Med |
| 114. | *Melilotus alba* Medik. ex Desr. | Leguminosae(s.l.)[3] | D | EUR | A | H | F | JL,FL | Med |
| 115. | *Melochia corchorifolia* L. | Sterculiaceae | D | TAM | A | H | U | JL,FL | Fo |
| 116. | *Mikania micrantha* Kunth. | Asteraceae | D | SAM | P | CL | U | TWF,MTF,JL,BAF | Med |
| 117. | *Mimosa diplotricha* Wright | Leguminosae(s.l.)[1] | D | SAM | P | SH | U | BAF,TWF | Med |
| 118. | *Mimosa pudica* L. | Leguminosae(s.l.)[1] | D | BRZ | P | H | U | TWF,MTF | Med |
| 119. | *Mucuna pruriens* (L.) DC. | Leguminosae(s.l.)[3] | D | TAF | P | CL | F | JL,FL | Food,Med |
| 120. | *Nicotiana plumbaginifolia* Viv. | Solanaceae | D | TAM | A | H | U | JL | Med |
| 121. | *Ocimum americanum* L. | Lamiaceae | D | TAF | P | H | F | JL | Med |
| 122. | *Opuntia vulgaris* Miller | Cactaceae | D | SAM | P | H | U | JL | Med |
| 123. | *Oxalis corniculata* L. | Oxalidaceae | D | EUR | P | H | U | JL | Food,Med |
| 124. | *Parthenium hysterophorus* L. | Asteraceae | D | NAM | A | H | U | TWF,MTF | Med |
| 125. | *Passiflora foetida* L. | Passifloraceae | D | SAM | P | CL | OR | FL | Orn |
| 126. | *Peltophorum pterocarpum* (DC.) K.Heyne | Leguminosae(s.l.)[2] | D | MAL | P | T | OR | TWF | Orn |
| 127. | *Peperomia pellucida* (L.) Kunth | Piperaceae | D | SAM | A | H | U | TWF | Med |
| 128. | *Physalis minima* L. | Solanaceae | D | TAM | A | H | U | TWF | Med |
| 129. | *Pilea microphylla* (L.) Liebm. | Urticaceae | D | SAM | A | H | U | TWF | Med |
| 130. | *Pistia stratiotes* L. | Araceae | M | TAM | P | H | U | WL | Orn |

Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 131. | *Plumeria rubra* L. | Apocynaceae | D | MEX | P | T | OR | TWF | Orn |
| 132. | *Portulaca oleracea* L. | Portulacaceae | D | SAM | A | H | F. | JL ,FL | Orn |
| 133. | *Pyrostegia venusta* (Ker Gawl.) Miers | Bignoniaceae | D | BRZ | P | CL | OR | FL | Orn |
| 134. | *Ricinus communis* L. | Euphorbiaceae | D | MED | P | SH | F | TWF | Food,Med |
| 135. | *Rorippa dubia* (Pers.) Hara. | Brassicaaceae | D | TAM | A | H | U | TWF | Unk |
| 136. | *Ruellia tuberosa* L. | Acanthaceae | D | TAM | A | H | U | TWF | Orn |
| 137. | *Saccharum spontaneum* L. | Poaceae | M | TWA | P | GR | U | FL | Fo,R,Th |
| 138. | *Scoparia dulcis* L. | Scrophulariaceae | D | TAM | A | H | U | FL | Med |
| 139. | *Senna alata* (L.) Roxb. | Leguminosae(s.l.)[2] | D | SAM | P | SH | OR | JL ,FL | Med,Orn |
| 140. | *Senna occidentalis* (L.) Link. | Leguminosae(s.l.)[2] | D | SAM | P | H | U | JL ,FL | Med |
| 141. | *Senna tora* (L.) Roxb. | Leguminosae(s.l.)[2] | D | SAM | A | H | U | JL ,FL | Med |
| 142. | *Sida acuta* Burm.f. | Malvaceae | D | TAM | A | H | U | FL | Med |
| 143. | *Solanum torvum* Sw. | Solanaceae | D | WI | P | SH | U | FL | Med |
| 144. | *Sonchus asper* (L.) Hill | Asteraceae | D | MED | A | H | U | TWF ,FL | Unk |
| 145. | *Sonchus oleraceus* (L.) L. | Asteraceae | D | MED | A | H | U | TWF | Unk |
| 146. | *Spermacoce hispida* L. | Rubiaceae | D | TAM | A | H | U | TWF | Med |
| 147. | *Spilanthes acmella* (L.) L. | Asteraceae | D | BRZ | A | H | U | FL | Med |
| 148. | *Stachytarpheta jamaicensis* (L.) Vahl | Verbenaceae | D | TAM | A | H | U | FL | Orn |
| 149. | *Stellaria media* (L.) Vill. | Caryophyllaceae | D | NAM | A | H | U | JL | Food,Med |
| 150. | *Strelitzia reginae* Banks | Strelitziaceae | M | SAF | P | H | OR | JL | Med |
| 151. | *Synedrella nodiflora* (L.) Gaertn. | Asteraceae | D | WI | A | H | U | JL | Med |
| 152. | *Tamarindus indica* L. | Leguminosae(s.l.)[3] | D | TAF | P | T | F | TWF | Food, Med |
| 153. | *Thuja occidentalis* Bailey | Cupressaceae | G | NAM | P | T | U | TEF | T |
| 154. | *Thunbergia alata* Bojer ex Sims | Acanthaceae | D | EAF | P | CL | OR | MTF | Orn, Med |
| 155. | *Tithonia diversifolia* (Hemsl.) A.Gray | Asteraceae | D | MEX, | P | H | OR | MTF | Med, Orn |
| 156. | *Tridax procumbens* (L.) L. | Asteraceae | D | CAM | A | H | U | TWF | Med |
| 157. | *Triumfetta rhomboidea* Jacq. | Malvaceae | D | TAM | A | H | U | TWF | Med |

ISSN: 2355-7079/E-ISSN: 2406-8195

Table 1. Continued

| S.No. | Accepted names | Families | Taxonomic Sub-group | Nativity | Habit | Growth form | Mode of introduction | Habitats invaded | Uses |
|---|---|---|---|---|---|---|---|---|---|
| 158. | *Typha angustifolia* L. | Typhaceae | M | TAM | P | GR | U | WL | Ht |
| 159. | *Ulex europaeus* L. | Leguminosae(s.l.)[3] | D | EUR | P | SH | U | FL | Orn |
| 160. | *Urena lobata* L. | Malvaceae | D | TAF | P | SH | U | JL | Med |
| 161. | *Xanthium strumarium* L. | Asteraceae | D | TAM | A | H | U | JL | Med, FU |
| 162. | *Youngia japonica* (L.) DC. | Asteraceae | D | SAM | A | H | U | FL | Med |
| 163. | *Eryngium foetidum* L. | Apiaceae | D | MEX | A | H | F | FL | Sp. |

**Abbreviations: Leguminosae (s.l.):-**Leguminosae (sensulato) includes all the three subfamilies (i.e., 1. Subfam. Mimosoideae, 2. Subfam. Caesalpinioideae, 3. Subfam. Papilionoideae);**Taxonomic Sub-group**: D:- Dicotyledons, M:- Monocotyledons, G:-Gymnosperms; **Habit** : P:- Perennial, A:-Annual**;Nativity:** TAM:-Tropical America, SAM:-South America, CAM:-Central America, TNM:-Tropical North America, TAF:-Tropical Africa, EAF:-Eastern Africa, NAM:- North America, SAF:-South Africa, WI:- West Indies, MEX:-Mexico, AUS:-Australia, EUR:-Europe, BRZ:-Brazil, MED:- Mediterranean; SWA= South West Tropical Asia**; Growth form**: H:-Herb, SH:-Shrub, GR:-Grass, SE:-Sedge, CL:-Climber, T:-Tree; **Mode of introduction**: U:-Unintentional, O:-Ornamental, F:-Food; **Habitats invaded**: TWF: - Tropical Wet Evergreen Forest, MTF:-Montane sub-tropical Forest, TEF-Temperate Forests, JL:-Jhumland, BAF:-Bamboo Forests, WL:-Wetland,FL:-Fallow Lands.**Uses** Med: medicinal, Man: Green manure, Bio: bioactive chemicals, Orn:ornamental,Sp:spice,T:timber,Ad:adulteration,Unk:unknown,Fo:fodder, R:rope making, Th: Thatching,Wwt :Waste water treatment, Ht: Hut thatch, FU: Biomass fuel, Bas: Basket Making.

# THE EFFECTIVENESS OF GLUCOMANNAN AND NANO ACTIVATED-CARBON AS HYPERCHOLESTEROL-LOWERING AGENTS

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Received: 25 April 2020, Revised: 29 October 2020, Accepted: 29 October 2020

THE EFFECTIVENESS OF GLUCOMANNAN AND NANO ACTIVATED-CARBON AS HYPERCHOLESTEROL-LOWERING AGENTS. Porang (konjac) plants have long been used as a food source and traditional medicine. Glucomannan derived from porang has been utilised for various uses such as antidiabetic and antihypercholesterolemia agent. This paper studies the mixture of porang flour and nano activated-carbon and its effect on the cholesterol activity of rats. The mixture of porang and activated carbon were subjected to test for male Sprague Dawley rats to test the antihypercholesterolemia activity. The result showed that concerted anticholesterol activity of porang and nano activated-carbon revealed the cholesterol level decreases in rat's blood. However, the different treatments of unleached and leached porang either leached porang and nano activated-carbon applied in the experiments showed that the levels of cholesterol decrease were slightly different (16–18%). Low glucomannan content as the alleged anticholesterol agent was regarded quite effective in lowering the cholesterol level in rat's blood and comparable with those of simvastatin which achieved 18% reduction. Therefore, it indicates potential utilisation as a functional food for a cholesterol-lowering agent. The involvement of activated carbon in the alleged anticholesterol agent (leached porang flour) did little in enhancing the cholesterol level decrease in rat's blood. The glucomannan in both leached porang flour and leached porang flour + nano activated-carbon shows potential utilisation as an anticholesterol agent. Yet, raw (unleached) porang is prospectively potential as a functional food for cholesterol-lowering.

Keywords: Porang, anticholesterol, nano activated-carbon, glucomannan

*EFEKTIVITAS GLUKOMANAN DAN NANO KARBON AKTIF SEBAGAI AGEN PENURUN KOLESTEROL. Tanaman porang (konjak) telah lama digunakan sebagai sumber makanan dan obat tradisional. Glukomanan yang berasal dari tanaman tersebut telah digunakan untuk berbagai kegunaan sebagai antidiabetes dan juga sebagai antihiperkolesterol. Tujuan dari penelitian ini adalah untuk menguji aktivitas kolesterol pada tikus melalui perlakuan kombinasi tepung porang dan nano karbon aktif. Kombinasi porang dan nano karbon aktif diuji pada tikus jantan strain Sprague Dawley untuk menguji aktivitas antihiperkolesterolemia. Hasilnya menunjukkan bahwa aktivitas antikolesterol porang dan nano karbon aktif menunjukkan penurunan tingkat kolesterol dalam darah tikus. Namun, perlakuan yang berbeda antara porang tanpa pencucian dan porang dengan pencucian dengan nano karbon aktif menunjukkan bahwa penurunan kadar kolesterol sedikit berbeda (16–18%). Kandungan glukomanan yang rendah dianggap cukup efektif dalam menurunkan kadar kolesterol dalam darah tikus, dibandingkan dengan kontrol positif (simvastatin); keduanya mencapai penurunan 18%; sehingga menunjukkan potensi pemanfaatannya sebagai makanan fungsional untuk menurunkan kolesterol. Pengaruh nano karbon aktif dalam meningkatkan aktivitas antihiperkolesterolemia (tepung porang yang dicuci) ternyata tidak banyak membantu dalam meningkatkan penurunan kadar kolesterol dalam darah tikus. Glukomanan dalam tepung porang yang dicuci dan tepung porang yang dicuci + nano karbon aktif menunjukkan potensi pemanfaatannya sebagai agen antihiperkolesterol. Namun, porang yang tidak dicuci berpotensi sebagai makanan fungsional untuk menurunkan kolesterol.*

*Kata kunci*: *Porang, antihiperkolesterol, nano karbon aktif, glukomanan*

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 http://dx.doi.org/10.20886/ijfr.2020.7.2.155-164 

## I. INTRODUCTION

*Amorphophallus muelleri*, locally called porang or *iles kuning*, is one of potential glucomannan source in Indonesia (Yanuriati, Marseno, Rochmadi, & Harmayani, 2017). They are prevalent as flour forms and could be used for various utilization, such as for food, industry, and health products. Porang plants are widely planted by the community and managed by Perum Perhutani across East Java, such as Jember, Nganjuk, Padangan, Saradan, Bojonegoro and Madiun. The total area of land reaches 1605.3 ha (Indriyani et al., 2010). Purified porang flour could be ingeniously converted into specific products for the manufactured (processed) items, such as specific type of food, including noodles, tofu and low-calorie artificial rice, a mixture for industrial raw materials, basic ingredients for film industries, and refreshment drink (Tester & Al-Ghazzewi, 2017). Chua et al. (2010) mentioned that glucomannan could afford potential health benefits, owing to its efficacious healing performances, such as anti-obesity activity, anti-hyperglycemic and hypercholesterolemia activities, laxative effect, prebiotic activity, and anti-inflammatory activity.

Nowadays, many people are very concerned about a healthy lifestyle. Besides, consumers are very interested in functional food associated with human health. Not only can such food serve as a source of energy and nutrition, but it should also perform physiological functions needed by the human body for maintaining their health. One of the potential functional foodstuffs is porang (konjac). This stuff product exhibits a high dietary fiber and being low in calorie (Directorate-General for Food Crops, 2013).

Consuming glucomannan which is prevalently contained in porang/konjac or depolymerised glucomannan, is remarkably good for maintaining human health. The mannans could provide several health benefits in the human body. The ability of mannans, especially konjac glucomannans, to prevent specific pathogens including *Escherichia coli* from adhering to the gut or bladder mucosa, particularly in the human body, is highlighted or put to thorough attention. Other benefits associated with the local gut environment are discussed together with more systemic effects in and on other organs. The glucomannans could be an option for therapeutic tools in curing a wide range of physiological disorders such as diverticulitis and Crohn's disease or ulcerative colitis (Tester & Al-Ghazzewi, 2013). Furthermore, a study by González-Torres et al. (2016) revealed that cholesterolemia was lower in glucomannan and glucomannan/spirulina treatment in rats. Glucomannan has been approved as generally regarded as safe (GRAS) food additive and had been approved by related agencies on food and health regulation in the USA, Canada and European Commission (Tester & Al-Ghazzewi, 2017).

On the other hand, nanotechnology has been applied to improve the effectiveness of drug delivery, movement, and efficacy in the human body. One of the nano products which have been used in the drug delivery system is nanocarbon. Nanocarbon derived from bamboo charcoal showed good performance in delivering *Eucommia ulmoides* extract with cumulative release rate was 70.67% and in vitro anticancer study of this nano carbon delivery system showed the inhibition rate against HCT116 cancer cells was 23% (Zeng, Zhang, & Huang, 2017). Carbon nanotubes have been used for medicine such as for chemotherapy drug delivery, for stem cell-related therapies, and thermal destruction of tumours (Shao et al., 2013). Furthermore, (Liu et al., 2010) said that carbon nanotube-based drug delivery had shown promises in 2 various *in vitro* and *in vivo* experiments including the delivery of small interfering RNA (siRNA), paclitaxel and doxorubicin. Meanwhile, the carbon activation using thermal or chemical method is known to create well-developed microstructures and porosity, as well as surface functionality ( Huang et al., 2018).

The utilisation of activated carbon nanoparticles exhibited efficient intracellular delivery of molecules while maintaining high

cells viability close to 100% (Sengupta, Kelly, Dwivedi, Thadhani, & Prausnitz, 2014). The prominent properties of nanocarbon, i.e. high specific area and porosity, high adsorption capacity and hierarchical porous structure, make them a good candidate as a carrier material (Huang et al., 2018). Among the carbonaceous materials, biomass-based activated carbon is attracting because of its unique internal structure having regularly interconnected mesopores, high surface area, low mass density, exceptional chemical stability, low cost and environmental friendly (Jain & Tripathi, 2015).

In this study, the synergy of porang flour, which emerged into nano activated-carbon derived from biomass was observed. The use of nano-activated carbon and porang flour which containing glucomannan as an anticholesterol agent is predicted to be able to enhance antihypercholesterolemia activity as tested in rat body as well as the implantation of glucomannan in the potential active site. This study aims to evaluate the concerted antihypercholesterolemia activity of porang flour and nano activated-carbon. The commercial antihypercholesterol (simvastatin) was used in this study as a comparison.

## II. MATERIAL AND METHOD

### A. Materials

Porang (*Amorphophallus muelleri*) pieces as originally already procured in chip shape were indigenous from East Java. Activated carbon was made from charcoal pine waste.

### B. Methods

#### 1. Preparation of Porang's Flour

Porang flour which was used in this study was generated from the species *Amorphophallus muelleri*. Porang flour was prepared based on our previous research. Porang tubers were sliced to make chip. The dried chip was ground to get the unbleached flour. Then unbleached flour was purified using ethanol 50% and $NaHSO_3$ 2% to get leached flour. (Pasaribu, Waluyo, Hastuti, Pari, & Sahara, 2016). Glucomannan content was measured based on Indonesia's National Standard (SNI 7939, 2013).

#### 2. Preparation of Activated Carbon

The nano activated carbon used in this experiment was prepared through the activation process from pine charcoal by a thermal method. The charcoal was activated in the retort at 800°C for 90 minutes (Pari, 1999). The activation intended mainly to remove the impurities (e.g. fat, residual high-boiling volatile matters, and impurities materials other than wood/lignocellulosic materials, such as dust, tiny sand particles, and dirt), thereby clearing its surface and enhancing its available pores numerously. Afterwards, as-prepared activated charcoal was ground mechanically; therefore, the size reduced vigorously to very tiny particles and denoted as nano activated-carbon. The resultant nano activated-carbon was characterised microscopically, and its properties were evaluated by refers to the Indonesian standard (SNI 06-7370, 1995).

#### 3. Combination of Porang and Nano Activated-Carbon

The nano-activated carbon and porang flour were mixed in the ratio of 90% (w/w) of porang flour and 10% of nano activated-carbon. The composition selection was made based on the results of previous studies which showed good results.

#### 4. Antihypercholesterolemia Activity Test

As many 42 healthy living male rats as of Sprague Dawley strain, which weighed 200 to 250 g were selected randomly; and then prepared for the *in-vivo* study for their exposure to the antihypercholesterolemia activity test. The appointed (selected) rats were divided into six groups, each comprising seven individual rats. The number of replication can be determined with Federer's Formula. For research with six treatment, the minimum number of replications were four rats. Those six groups were further assigned each for six different allocations concerning the activity test of antihypercholesterol agents (Table 2),

which consisted of consecutively (1) normal group (the group of test animals that were not given treatment from the beginning to the end of the study); (2) positive control group (simvastatin); (3) negative control group (aqua dest); (4) group treated with porang aquadest before leaching-treatment (unleached porang flour, supposedly with lower glucomannan content), (5) group treated with porang flour after leaching treatment (leached porang flour, supposedly with higher glucomannan content); and (6) group treated with the mixture of leached porang flour (90%) + nano-activated carbon (10%).

The appointed rats in each group were allowed to acclimatise themselves for two weeks before the antihypercholesterolemia activity test started. During the acclimatisation, the animals were each given daily a standard feed as much as 20 g and drinking water *ad libitum*. Afterwards, those acclimatised rats also underwent the so-called induction phase of hypercholesterolemia, which was performed with high-fat feeding (7.5% egg yolks, 7.5% goat fat, 85% standard diet). The induction phase lasted for four weeks or proceeded until the rats revealed their blood cholesterol levels more than 200 mg/dL. Before the induction-phase treatment (immediately after the 2-week acclimatisation duration), those appointed rats were checked for their blood cholesterol levels using a strip test of the so-called Easy touch® and CHOD-PAP cholesterol kit, Biolabo®. Before the cholesterol-level checking, those rats should undergo the fasting period (without eating and drinking) for more or less (±) 16 hours. After cholesterol checking, the induction process could begin. Further, after the induction, those inducted rat animals were ready for anti-hyper-cholesterolemia activity test that comprised those six allocated treatments (Table 2).

That anticholesterol test was performed on the inducted rats for five weeks, whereby the rats in each group were treated (infected) with any of the supposedly anticholesterol agents (i.e. unleached porang flour; leached porang flour exclusively; and the mixture of leach flour and nano activated-carbon) (Table 2); and the anticholesterol dosages for each group (designated as F1, F2, and F3) were similar (350 mg/kg BW). While undergoing the anticholesterol test, concurrently all those tested rats also underwent the oral feeding treatment, whereby the rats in each group was fed with high-fat diets, except for the normal group (Table 2). One week after the start of anticholesterol treatment, each of the treated rats was checked for their cholesterol level using Easy touch ®'s strip test. When the five-week duration for anticholesterol test treatment ended, immediately, the blood of all treated rats was taken to obtain the serum. The obtained serum was used as a sample for measuring the rat's total cholesterol level.

This research that used animal (rats) as a model of a sort was assessed and finally approved by the ethics committee of Bogor Agricultural University under the registered IPB No. 45-2017. Moreover, the experimental procedures with rat objects were in strict accordance with animal ethical committee guidelines as stipulated for the care and use of laboratory animals.

### C. Analysis

The data was analysed using descriptive qualitative method. The characteristic activated carbon resulted from an average of three replications. The percentage of cholesterol decrease resulted from the average of reduction at one week after treatment and final treatment.

## III. RESULTS AND DISCUSSION

### A. Porang's Flour Characteristics

Characteristics of porang flour generally can be evaluated by its moisture content, ash content, protein content, fat content, and carbohydrate levels. The important data for this research is glucomannan content. Glucomannan content in porang flour after the leaching treatment was 83.96%; confirmed that leaching process using ethanol 50% and $NaHSO_3$ 2% effectively increased the glucomannan content compared to the initial content prior leaching (32.65%).

The glucomannan content of leached porang flour was 83.96%. This value was much higher than the glucomannan content of the previous study conducted by the same leaching treatment (Pasaribu et al., 2016). The glucomannan content of porang flour also was higher than konjac flour extracted from *Amorphopallus guripingensis* (61.35%) and *Amorphopallus rivirei* (72.80%). They used 50% of ethanol, followed by further leaching using absolute ethanol at 40ºC and centrifugation (Huang et al., 2016). Meanwhile, other study revealed that glucomannan could be simply extracted by centrifugation in 1500 g for 15 minutes at 35ºC and 75ºC generated glucomannan contents of 32 g/100 g and 35 g/100 g, respectively (Tatirat & Charoenrein, 2011). However, a glucomannan content of this study was lower but still comparable with the result of another study which used $Al_2(SO_4)_3$ and ethanol for purification (Yanuriati et al., 2017). Besides, the temperature of extraction also affected the quality of konjac flour. Extraction of glucomannan using 40% ethanol at 80ºC yielded glucomannan content about 90%, the ash content 0.01%, transparency 58%. Therefore, the temperature effect was favourable to improve konjac glucomannan purity (Xu et al., 2014).

## B. Characteristic of Nano-Activated Carbon Material

Characteristics of nano-activated carbon are presented in Table 1. At general, all aspects of nano-activated carbon's properties were within the acceptance limit of Indonesian standard. The Iod number of obtained nano-activated carbon was much higher from the minimum limit of standard.

Microscopic observation of nano-activated carbon material was performed using scanning electron microscopy (SEM) and the results, as shown in Figure 1. SEM images revealed that the mechanical grinding of activated carbon yielded an irregular form of nanocarbon. However, the mechanical disintegration applied in this study has successfully degraded the size of charcoal into nano-scale (Figure 1).

The characteristics of nano activated-carbon (derived from pine charcoal) concerning its moisture content, ash content, volatile matters, and iod number were in acceptance limit of those as stipulated by the Technical Active Charcoal Standard or Indonesia's National Standard (SNI 06-7370-1995) (Standar Nasional Indonesia, 2013). Activated carbon consists of functional groups bonded to fused-aromatic rings, which would be expected to possess chemical properties similar to those in the aromatic hydrocarbons. Surface functional groups on the carbon matrix can be manipulated and adjusted by thermal, hydro-thermal or chemical treatments for particular functions (Ao et al., 2018).

The high iod number of obtained nano activated-carbon was attributed to the pores structure change during the activation. As the activation time increases, micropores develop at the initial stage of activation. At the second stage, the micropores and the mesopores develop together, and at the third stage, the

Table 1. Characteristic of activated charcoal as the nano-activated carbon material

| Item | Characteristics*) | | | | | |
|---|---|---|---|---|---|---|
| | Moisture content (%) | Ash content (%) | Volatile matter (%) | Fixed carbon (%) | Degree of crystalinity (%) | Iod number (mg/g) |
| Nano activated pine charcoal **) | 13.11 | 4.90 | 3.06 | 92.04 | 49.51 | 843.35 |
| SNI 06-7370-1995 (for active carbon) | Max.15 | Max.10 | Max.25 | Min.65 | - | Min.750 |

Remarks: (-) not specified; Max = maximum; Min = minimum; *) the result from an average of 3 replications; **) interchangeably in this aspect could be regarded as nano activated carbon material

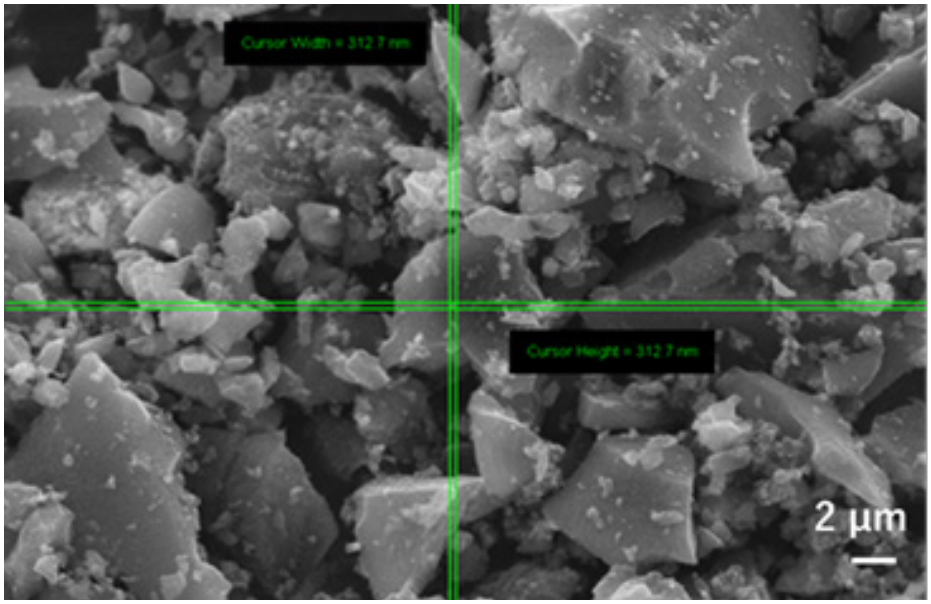


Figure 1. SEM image of nano-activated carbon from pine charcoal (2500x magnification)

mesopores mainly develop with a decrease of the micropores (Lee et al., 2019). The increasing micropores surface area of the activated carbon, the adsorption capacity also increases (Ahmed et al., 2019). With rising temperatures, the porous structures on the surface of the carbon materials and the specific surface area increase, but breakdown and disintegration of the porous structure can happen if the temperature rises too high (Wan & Hu, 2019). Such degradation and destruction consequently induced the increase in amorphous regions of the activated charcoal, while concurrently caused a decrease in its crystalline regions. In this regard, the crystallinity degree in woods as well as lignocellulose materials, either carbonised, activated, or not, represents the ratio between the crystalline region and the overall region (crystalline + amorphous).

Figure 1 revealed the small fragments and 3D structure with the large specific surface area were obtained after activation at 800ºC, and it is in good accordance with the previous study which conducted activation using KOH followed by carbonisation at 850ºC for 2 h (Awasthi et al., 2019). The high iod number also associated with the adsorption sites of activated carbon. The adsorptive properties of such biomass-derived activated carbons have been utilised as pollution control materials for control of polluting gases (llingworth, Rand, & Williams, 2019). Activated carbon concept includes several different carbonaceous materials with various structures, forms and size of pores (Volperts et al., 2019). However, the properties of the resulting activated carbon can be influenced by types of activating reagents, activation time, impregnation condition, carbonisation temperature, inorganic impurities and others (Yahya, Al-Qodah, & Ngah, 2015). Besides, the chemical constituents of lignocellulosic materials such as cellulose, hemicellulose and lignin also could influence the properties of activated carbon. Based on the other study, lignin can be considered as being the major contributor of all chars and activated carbons properties (Cagnon et al., 2009).

### C. Antihypercholesterolemia Activity Test

The anticholesterol performance was represented by the total cholesterol level and its decrease in rat's blood, as presented in Table 2.

Antihypercholesterolemia activity test revealed that the use of porang flour, which containing glucomannan was sufficient to decrease total cholesterol level in rat's blood

(Table 2). The decreases (in percentage) of overall cholesterol level for the three groups of treatments, which allegedly as anticholesterol agents, were consecutively 18% (for unleached porang flour/F1, containing low glucomannan), 16% (for leached porang flour / F2, containing high glucomannan), and 17% (for leached porang flour that contained high glucomannan, mixed with nano activated-carbon/F3), whereby each of those groups used the same dosages of the alleged anticholesterol agents (350 mg/kg BW).

In the normal group, there was an increase in cholesterol levels. This is thought to be caused by the inadequate 0.01% PTU solution mixed in drinking water which causes the initial condition of hypercholesterolemic rats to be not maximal. The initial cholesterol of the treatment is still below 100 mg/dl.

By analysing the results from Table 2, the three groups of alleged anticholesterol agents (F1, F2, F3) were seemed to have similar efficacious effect in decreasing the cholesterol level in rats' blood (16–18%) (Table 2). However, the use of porang flour containing glucomannan performed more effective in the total level of cholesterol than the negative control (14%). Furthermore, the performance of the alleged anticholesterol agent, represented by F1 group, had a similar percentage of total level cholesterol with the positive control group (using simvastatin as anticholesterol agent) after five weeks of observation. Both are exerting the same cholesterol decreases by about 18% (Table 2). Simvastatin is the common cholesterol drug used by the community and commercially available.

From the resulting related quantitative figures (Tables 2), it revealed that unleached porang flour/F1 (with 32.65% glucomannan content) afforded the cholesterol level decrease (18%), which was not far different from the

Table 2. Total cholesterol level and total cholesterol level decrease in rats' blood due to the antihypercholesterolemia activity test treatment

| Groups allocated for the treatment | Antihypercholesterolemia activity test treatment | | |
|---|---|---|---|
| | Total cholesterol level, one week after the treatment (mg/dl) | Total cholesterol level, at final week of treatment (5 weeks)(mg/dl) | Cholesterol decrease level (%) |
| Normal group | 70.25±2.99 | 85.25±9.07 | (21.47±13.10) |
| Positive control / simvastatin (C+) | 94.25 ± 16.94 | 76.00 ± 11.17 | 18±12.45 |
| Negatif control (C-) | 76.75± 7.63 | 66.00±7.16 | 14±5.01 |
| Unleached porang flour / without leaching (proved with low glucomannan content)[1)], dose 350 mg/kgBW (F1) | 79.75±3.20 | 65.75±9.00 | 18±10.95 |
| Leached porang flour (proved with high glucomannan content)[1)], dose 350 mg/kgBW (F2) | 70.00±4.32 | 58.75±5.5 | 16±11.22 |
| Leached porang flour (proved with high glucomannan content)[1)] (90%) + nano active-carbon (10%)[2)], dose 350 mg/kgBW (F3) | 80.25±10.01 | 67.50±16.52 | 17±11.57 |

Remarks: the antihypercholesterolemia activity test treatment lasted for five weeks; [1)] please refer to Table 1; [2)] interchangeably regarded as nano activated pine charcoal; F1, F2, and F3 considered to be the tested anticholesterol agents

leached porang flour with leaching/F2 (with 83.86% glucomannan) that decreased the cholesterol level decrease about 16%. The percentages of cholesterol decreased was not far different either the leached porang flour (mixed with active carbon)/F3 that decreased the cholesterol level up to 17%. This results suggested that raw porang flour (containing relatively low glucomannan; without leaching) was quite effective in lowering the total cholesterol level in rats' blood.

The study also assesses the involvement of activated carbon (with its remarkable adsorption capacity) as an anticholesterol agent by combining it with glucomannan. The important physical properties of activated carbon are drug-loaded and wettability. The effectivity of activated carbon influences those as a carrier (Miriyala, Ouyang, Perrie, Lowry, & Kirby, 2017). In this study, the moisture content of nano-activated carbon was 13%. This water might affect the effectiveness of glucomannan as a cholesterol-lowering agent (Malik, 2013).

The other important properties of activated nanocarbon on drug release or delivery system are associated with dissolution and diffusion from the system simultaneously (Mondal, Hoang, Manivasagan, Kim, & Oh, 2019). Furthermore, the size and the shape of activated nanocarbon also might influence the effectiveness of glucomannan delivery to specific sites as an anticholesterol agent. Based on the other reported study, the promising drug delivery into diseased sites can be achieved through spherical-shaped nanoparticles with a sub-50 nm diameter (Yallappa, Abdul Manaf, & Hegde, 2018). The morphology of pine activated charcoal in this study was nano-sized, although not uniform in size.

The related research performed by (Yelaware Puttaswamy & Urooj, 2016) showed the notable performance of mahogany extracts in lowering the cholesterol level in rats' blood. They asserted that aqueous mahogany extracts (using water solvent) which was fed to the rats as much as consecutively 250 mg kg -1 BW and 500 mg $kg^{-1}$ BW and performed cholesterol level lowering by 38.80% and 41.04%, respectively. These values were more significant than the cholesterol level lowering value achieved by the positive control (satin), which was 37.31%. However, the cholesterol-lowering level of this study was lower, which were ranged from 16–18%. Furthermore, the effectiveness of glucomannan as an anticholesterol agent in this study was comparable with the capacity of *Vitis vinera* extracts which could reduce the cholesterol level in 10–21% (Devi & Singh, 2017).

## IV. CONCLUSION

The percentage of glucomannan and nano activated-carbon in lowering cholesterol (17%) level was not different even slightly lower than the treatment of glucomannan only (18%). The leaching pre-treatment on konjac flour did not affect the effectiveness of glucomannan in lowering cholesterol level. The presence of the porous structure of activated carbon has been supposed to be used as a potential substance/ drug carrier. In contrast, the physical properties of activated carbon might be very influential to its performance.

## ACKNOWLEDGEMENT

The authors in this occasion wish to express sincere gratitude, convey high appreciation and feel deeply indebted to the Forest Product Research and Development Center, the Ministry of Environment and Forestry of the Republic of Indonesia, which have supported this research and provided the related facilities and other beneficial items.

## AUTHORS' CONTRIBUTIONS

GP (Gunawan Pasaribu) conceived the study, and inspired his idea to the research entities, especially the research's main cores; GP, TK (Totok K Waluyo), GPi (Gustan Pari), and NH (Novitri Hastuti) designed and carried out the experiments; GP, TK, GPi and NH wrote the manuscript. GP serves as the main author.

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## AUTHORS INDEX

## KEYWORDS INDEX

# GUIDELINE FOR AUTHORS

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## AIM AND SCOPE

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## ACKNOWLEDGEMENT

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