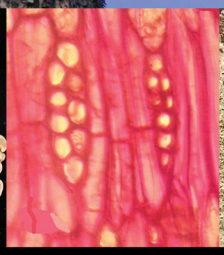
# Bijaysal

## A Monograph of *Pterocarpus marsupium* in Nepal





**Government of Nepal**
**Ministry of Forests and Environment**
**Department of Plant Resources**
**National Herbarium and Plant Laboratories**
**Godawari, Lalitpur, Nepal**

**2021**

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## "A Monograph of *Pterocarpus marsupium* in Nepal"


**Editors:**

**Lajmina Joshi**

**Sangeeta Rajbhandary**

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TLS of wood (Pratikshya Chalise),
Seedling (Ram Krishna Bhandari),
Anti-microbial test (Pramesh Bahadur Lakhey),
Wooden Tumblers (Dipesh Pyakurel).

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# FOREWORD

*Pterocarpus marsupium* Roxb, locally known as "Bijaysal" is a medium to a large-sized deciduous tree belonging to the family Fabaceae. The species is native to India, Nepal, Bangladesh, Sri Lanka, and Taiwan. In Nepal the species is confined to an altitude of 100 to 640 m. The plant has a limited global distribution and due to its multipurpose uses, especially being highly exploited for the extraction of Kino gum, the trees are disappearing rapidly. The aqueous extract of its heartwood is considered as a miracle cure against diabetes and several other uses are mentioned in traditional systems of medicine.

Being one of the prioritized species Government of Nepal has banned its cutting, collection, transportation and export for commercial purpose. Recently, the Department of Forests has prepared "*Bijaysal Conservation Action Plan 2018*" for the conservation of Bijaysal and is the first conservation action plan for plant species in Nepal. Therefore, assessing the detailed information on its taxonomy, reproductive biology, ecology, distribution, conservation status of the species and developing an accurate species level identification through its anatomical study is of utmost importance. Owing to the existing threats as well as poor seed viability, slow growth and poor natural regeneration of this species; hunting for effective measures to enhance the seed germination is also essential.

I am delighted to see the publication "*Bijaysal: A monograph of Pterocarpus marsupium in Nepal*" and congratulate all the contributors of the manual, for their significant contributions. The book includes detail information on taxonomy, reproductive biology, anatomy, distribution, ecology, population status, germination, ethnobotany, phytochemistry, pharmacology, anti-microbial activity, threats, conservation and trade of Bijaysal. I would also like to express my sincere thanks to the editors- Lajmina Joshi, Former Scientific Officer, Department of Plant Resources; Prof. Sangeeta Rajbhandary, Central Department of Botany, Tribhuvan University; Buddi Sagar Paudel, Spokesperson, Ministry of Forests and Environment; Sanjeev Kumar Rai, Director General, Department of Plant Resources; and Subhash Khatri, Chief, National Herbarium and Plant Laboratories for their support and effective coordination and hard work to bring this manual. I am also thankful to Shamik Mishra for the Nepali translation of the relevant chapters of the book. I would like to thank all the staffs of Department of Plant Resources, especially the staffs of National Herbarium and Plant Laboratories, Godawari for their direct as well as indirect contribution to bring out this book. I strongly believe that the information generated in the book will be helpful to the researchers, local stakeholders, community forests, private nurseries as well as the general public who have a keen interest towards the target species and will ultimately help in proper identification, management, sustainable utilization and conservation of existing populations of Bijaysal.

July, 2021

Dr. Pem Narayan Kandel
Secretary
Ministry of Forests and Environment
Singhadurbar, Kathmandu

# PREFACE

*Pterocarpus marsupium* commonly known as "Bijaysal" in Nepal is a deciduous tree in the family Fabaceae. The aqueous extract of its wood was used against diabetes since very long time and the species has been highly exploited for the heartwood as well as for the Kino gum. Poor natural regeneration, poor seed viability and germination, grazing, over exploitation of the tree have resulted massive decrease in natural populations worldwide. The IUCN Red List of Threatened Species has enlisted the tree in 'Near Threatened' category. Government of Nepal has declared Bijaysal as a protected species and has banned its collection, fetching and transportation for commercial purpose. A wide diversity of chapters that cover information on almost every aspect of Bijaysal have been incorporated in the monograph.

We believe the book will be helpful to the scientific community, local stakeholders as well as general public and will help in its proper identification, management, sustainable utilization and conservation.

It is a team work, but still we would like to specially thank Pratikshya Chalise, Research Officer, National Herbarium and Plant Laboratories, Godawari, for her contribution in different chapters and all necessary requirements to bring out this book.

We would specially like to thank the contributing authors Mohan Dev Joshi, Secretary, Ministry of Industry, Tourism, Forests and Environment, Karnali Province, Surkhet; Pashupati Nath Koirala, Ministry of Forests and Environment, Singhadurbar, Kathmandu; Sajita Dhakal, National Herbarium and Plant Laboratories, Godawari; Parasmani Yadav, Devi Prasad Bhandari from Natural Products Research Laboratory, Thapathali; Pramesh Bahadur Lakhe and Sachita Joshi from Department of Plant Resources, Thapathali; Ram Krishna Bhandari from Plant Research Centre, Nepalgunj, Banke; Prof. Suresh Kumar Ghimire and Neelam Pandey from Central Department of Botany, Tribhuvan University; Dipesh Pyakurel from Resources Himalaya Foundation, Sanepa and Yagya Raj Paneru, Capital College and Research Centre, Koteshwor.

Several data were collected from the field by the authors to complete the chapters, so we take this opportunity to thank Yagya Raj Paneru; Ghanashyam Chalise, Charpala Community Forests User Group, Rupandehi; Parbati Chalise; Prabhat Chalise; Pratima Chalise; Bashu Dev Bhatt; Parwati Bhatt for helping during the sample collection from different places of Rupandehi, Kailali and Kanchanpur districts and during ethnobotanical survey at Kailali and Kanchanpur districts. The local at Malakheti, Khamaura, Kailali district and Bhimdattanagar, Kanchanpur district are especially acknowledged for their warm hospitality and knowledge. Dambar Karki, District Plant Resource Office, Kailali; Maheshwori Bhatt, Assistant Forest Officer,

Division Forest Office, Kanchanpur; Bodh Raj Subedi, Divisional Forest Officer, Division Forest Office, Rupandehi; and Maha Laxmi Sharma, Assistant Forest Officer, Division Forest Office, Rupandehi for their direct as well as indirect help during the field visits and sample collection. We are greatly indebted to the entire management committee of Charpala CFUG and Shankarnagar CFUG, Rupandehi and Gwalabari CFUG, Bani, Kanchanpur for their direct as well as indirect help during the visits to the respective community forests.

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Photo credits are provided to these persons: Pratikshya Chalise, Yagya Raj Paneru, Ghanashyam Chalise, Ganga Datta Bhatt, Amrit KC, Ram Krishna Bhandari, Neelam Pandey, Dipesh Pyakurel and Pashupati Nath Koirala.

We are highly obliged to Shamik Mishra for the Nepali translation of the relevant chapters of the book. We acknowledge Mahesh Maharjan for the current layout and printing.

**- Editors**

# ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| °C | degree Celsius |
| CAMP | Conservation Assessment and Management Prioritization |
| cm | centimetres |
| CF(s) | Community Forest(s) |
| CFUG | Community Forests Users Group |
| DoF | Department of Forests |
| DPR | Department of Plant Resources |
| *et al*. | 'et alia', 'and others' |
| FAA | Formalin- Acetic Acid- Alcohol |
| gm | gram |
| GoN | Government of Nepal |
| GoI | Government of India |
| hrs | hours |
| IKI | Iodine- Potassium Iodide |
| IUCN | International Union for Conservation of Nature and Natural Resources |
| KATH | National Herbarium and Plant Laboratories, Lalitpur, Nepal |
| mg/kg | miligram per kilogram |
| mm | milimetre |
| $mg.ml^{-1}$ | miligram per mililitre |
| µg | microgram |
| µg/ml | microgram per ml |
| µm | micrometer |
| nm | nanometre |
| RLS | Radial Longitudinal Section |
| SF | Stomatal Frequency |
| SI | Stomatal Index |
| Subsp. | Sub-species |
| TTC | Tetrazolium Tetrachloride |
| TS | Transverse Section |
| TLS | Tangential Longitudinal Section |
| TUCH | Tribhuvan University Central Herbarium |
| VS | Vertical Section |

# CONTENTS

# CHAPTER - 1
# INTRODUCTION

**Pratikshya Chalise and Subhash Khatri**

Fabaceae or Leguminosae with about 751 genera and about 19,000 known species, is the third largest family of flowering plants after Orchidaceae and Asteraceae and is one of the economically important family of flowering plants (Xu and Deng, 2017; Doyle, 2001). It is a very diverse family that includes a wide variety of growth forms, including trees, shrubs, herbaceous plants and some vines or lianas; ranging from tiny alpine ephemerals to large trees in the tropical rainforest canopies (Doyle, 2001). Economically, Leguminosae is considered as the second most important family after Poaceae. Majority of the members of this family are important due to their food value, fiber extraction, production of dyes and have also been used as natural fertilizers since ages. This is because the members of this family are characterized by the presence of root nodules that contain nitrogen-fixing bacteria (Xu and Deng, 2017) except in the genus *Styphnolobium*.

## Genus Pterocarpus

*Pterocarpus* is a deciduous tree belonging to the family Fabaceae. The members of this genus are characterized by the presence of compound leaves with alternate leaflets; yellow flowers arranged in racemes; flat, orbicular, indehiscent pods surrounded by circular wing, with one to two dolabriform seeds (Troup, 1921). The barks are greyish brown in colour which peels off in irregular scales and bright red gum resin is exuded when slashed (Troup, 1921; Gamble, 1922; Badakhane *et al.,* 2010; DoF, 2018).

They are renowned for the "Padauk wood" which is obtained from several species of the genus *Pterocarpus* (Pullaiah, 2019(b)). But, Troup (1921) considers only three species namely; *P. macrocarpus* (Myanmar Padauk or, the True Padauk), *P. dalbergioides* (Andaman Padauk) and *P. indicus* (Malay Padauk or, Indian Padauk) as the actual "Padauk". All padauks are of African or Asian origin and are valued for their toughness, stability, durability and decorativeness, most having a reddish wood (Azamthulla *et al.,* 2015).

Most *Pterocarpus* woods contain either water or alcohol-soluble substances and can be used as dyes; which is used to colour food, alcoholic beverages, wood polish, metal varnishes, textiles, wool, silk, leather, jute, anti-sun tanning agents, hair dyes, medicine coating and as dye in sensitized solar cells (Pulliah and Reddy, 2019).

**List of species**

The genus is pan-tropical in distribution, but the number of species has been mentioned differently by various researchers. Allen and Allen (1981) reported 60-70 species within the genus *Pterocarpus*. Later, Hooker (1879) as well as Brandis (1907) reported the occurrence of 15 species. However, Gamble (1922), Troup (1921), Pearson and Brown (1932) mentioned the occurrence of 35 species in the tropics and subtropics world-wide. Similarly, The Plant List has included 235 scientific plant names of species rank and 33 infraspecific taxa for the genus *Pterocarpus*. Out of which, 72 are the accepted names, 155 are synonyms, two are misapplied names and 39 names are unresolved names (http://www.theplantlist.org/tpl1.1/search?q=pterocarpus, retrieved on 02-03-2021). However, The Plants of the World online has mentioned the occurrence of 41 species within the genus *Pterocarpus* (http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:331884-2, retrieved on 02-03-2021). However, Roskov *et al.* (2020) have reported 35 species and seven subspecies existing worldwide except in Australia; with greatest diversity in Africa (https://www.catalogueoflife.org/data/taxon/74N7, retrieved on 02-03-2021).

1. *Pterocarpus acapulcensis* Rose
2. *Pterocarpus albopubescens* Hauman
3. *Pterocarpus amazonum* (Benth.) Amshoff
4. *Pterocarpus angolensis* DC.
5. *Pterocarpus antunesii* (Taub.) Harms
6. *Pterocarpus brenanii* Barbosa and Torre
7. *Pterocarpus claessensii* De Wild.
8. *Pterocarpus dalbergioides* DC.
9. *Pterocarpus echinatus* Pers.
10. *Pterocarpus erinaceus* Poir.
11. *Pterocarpus gilletii* De Wild.
12. *Pterocarpus hockii* De Wild.
13. *Pterocarpus homblei* De Wild.
14. *Pterocarpus indicus* Willd.
15. *Pterocarpus lucens* Guill. and Perr.
16. *Pterocarpus macrocarpus* Kurz
17. *Pterocarpus marsupium* Roxb.
18. *Pterocarpus mildbraedii* Harms
19. *Pterocarpus mutondo* De Wild.
20. *Pterocarpus officinalis* Jacq.
21. *Pterocarpus orbiculatus* DC.
22. *Pterocarpus osun* Craib
23. *Pterocarpus rohrii* Vahl
24. *Pterocarpus rotundifolius* (Sond.) Druce
25. *Pterocarpus santalinoides* DC.
26. *Pterocarpus santalinus* L.f.
27. *Pterocarpus soyauxii* Taub.
28. *Pterocarpus ternatus* Rizzini
29. *Pterocarpus tessmannii* Harms
30. *Pterocarpus tinctorius* Welw.
31. *Pterocarpus velutinus* De Wild.
32. *Pterocarpus villosus* (Benth.) Benth.
33. *Pterocarpus violaceus* Vogel
34. *Pterocarpus zehntneri* Harms
35. *Pterocarpus zenkeri* Harm.

Most of these species are found in Africa, notably in Nigeria, Cameroon, Sierra Leone and Equatorial Guinea (Pulliah, 2019(b)). However, only one species is found in Nepal (https://www.efloras.org, retrieved on 02-03-2021).

**Pterocarpus marsupium Roxb.**

*Pterocarpus marsupium* Roxb., popularly known as "Bijaysal" is a deciduous tree that grows up to 33 meters high (Barstow, 2017). It was first reported from Circar Mountains in "*Plants of the Coast of Coromandel, Volume 2*" by William Roxburgh from East India Company during 1798 which was officially published in 1799. It was locally called as "Yangshaw of the Telingas" (Roxburgh, 1799).

*Pterocarpus marsupium* is a repository of numerous bioactive compounds and possesses medicinal properties. The wooden tumbler (Figure 1d) made up of its heartwood is used for drinking water as traditional remedy because of its medicinal property (Badakhane *et al.,* 2010; Joshi *et al*., 2012). Since long time, it has been used in the treatment of krmiroga (worm infection), kustha (leprosy), prameha (diabetes), pandu (anaemia), medodosa (obesity) as well as several other cases (GoI, 2013). It is popularly known for a red gum resin (Kino) that exudes from the cuts and injuries (Figure 1a) in the bark (Badakhane *et al.,* 2010), which gets denser and appears blood red when it comes in contact with air (Figure 1c). Bark is often slashed to collect the Kino gum, which is an important remedy against diabetes (Khanal and Bhattarai, 2020). This species has medicinal, fodder as well as timber values. It has superlative characteristics in its wood and has high medicinal value so it is considered as one of the most prioritized woods for millennia (Badakhane *et al.,* 2010).

The plant is an important tropical tree with a restricted population existing within an altitudinal range of 100 to 640 m, predominantly in Western tarai compared to Central and Eastern Nepal. IUCN Red list of threatened species has enlisted it as a 'Near Threatened' tree because of its declining population globally (Barstow, 2017). Considering its limited distribution and declining population in Nepal, GoN has also prioritized this species as an important floral component. Thus, to increase the population of Bijaysal through both *in-situ* and *ex-situ* conservation (Figure 1b) and to sustain its healthy population in natural habitats, the "*Bijaysal Conservation Action Plan 2018-2022*" has also been prepared (DoF, 2018).

Bijaysal should have been a matter of concern to ecologists, forest scientists and environmentalists, but comparatively very few works have been carried out regarding this species in Nepal. Although GoN has prioritized this species for its conservation, only few initiatives have been taken by Community Forest User Groups and other stakeholders, therefore the conservation approaches need to be intensified. Restricted distribution patterns, low germination and slow growth, long juvenile period and tough access to mature flowering trees might have been the reasons for its limited study (DoF, 2018; Pulliah, 2019(b)). To conserve the existing populations of Bijaysal, we need to control illegal cutting and fetching, unsustainable harvesting, habitat destruction, and trade inside as well as outside the country. Together, appropriate

measures should have to be adopted to promote natural regeneration, propagation as well as plantation of Bijaysal in the lowlands of Nepal.


**Figure 1:** *Pterocarpus marsupium* **a.** Trunk showing deep cut for collection of Kino gum; **b.** Conservation initiatives taken at Gwalabari CFUG for raising public awareness; **c**. Kino gum exudating from the bark; and **d.** Wooden tumblers made from the heartwood of Bijaysal. (**Photos:** a. and c. Yagya Raj Paneru; b. and d. Pratikshya Chalise).

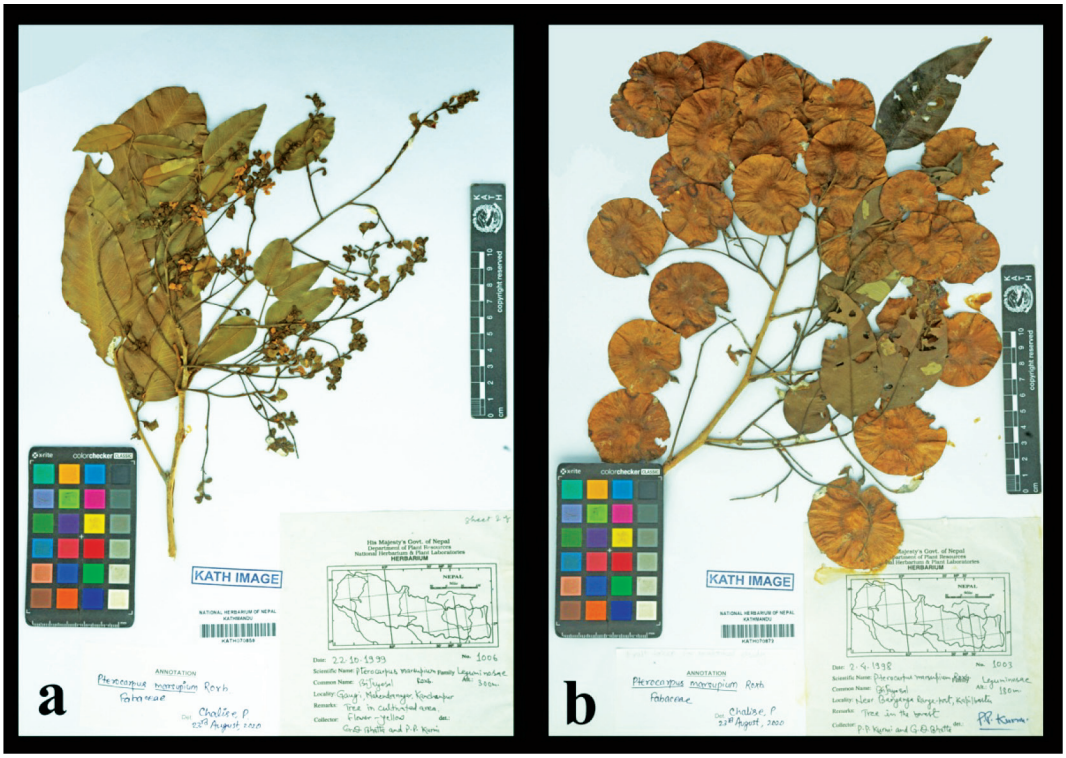


**Figure 2:** Digitized herbarium specimens of *Pterocarpus marsupium* Roxb., housed at KATH. **a.** Flowering state; and **b.** Fruiting state.

# CHAPTER - 2
# TAXONOMY

**Pratikshya Chalise**

## Scientific and Vernacular names

**Scientific name:** *Pterocarpus marsupium* Roxb., Pl. Coromandel 2(1): 9, t. 116 (1799).
**Nepali:** Bijayashal, Bijaysal, Vijaysar, Bijayasar, Banduk pushp.
**English**: Gum Kino, Indian Kino Tree, Malabar Kino Tree, East Indian Kino
**Ayurveda:** Biyo, Asana, Vijaysar, Pitasara, Asanam, Bijasal, Bijayasar
**Sanskrit:** Bijasra, Pitasara, Bijaka, Asanaka

## Etymology

The genus *Pterocarpus* is derived from two Greek words "pteron" meaning "winged" and "karpos" meaning "fruit" referring to the winged pod present in the members of this genus (Hutchinson, 1964; Pulliah, 2019(b); Silva *et al.,* 2019). Similarly, "marsupium" refers to the "pocket in female plants for storing reproductive structures i.e, seeds". Thus, *Pterocarpus marsupium* is a tree with winged fruit that enclose the seeds. Some literatures also refer the specific epithet to describe the distribution of this tree in specific pocket areas (Duthie, 1915).

## Taxonomy and Systematic position

Kingdom - Plantae
Phylum - Tracheophyta
Class - Magnoliopsida
Order - Fabales
Family - Fabaceae
Genus - *Pterocarpus* Jacq.
Species - *P. marsupium* Roxb.

The generic name "*Pterocarpus*" was given by Linnaeus in 1754. It was later described by several researchers like Hooker (1879), Brandis (1907), Troup (1921) and Brummitt (1992). However, the taxon "*Pterocarpus marsupium*" was first described by William Roxburgh in 1799 from the Circar mountains, Coromandel in "*Plants of the Coast of Coromandel: selected from drawings and description presented to the court of the East India Company*" volume II (Roxburgh, 1799).

Polhill (1981) categorized two groups within Leguminosae, based on the nature of fruits; winged fruits versus unwinged fruits, and placed the genus *Pterocarpus* in the

tribe Dalbergieae. Similarly, Wu and Raven (2010) in Flora of China volume 10 and Pulliah (2019(b)) also placed the genus *Pterocarpus* within the tribe Dalbergieae of the family Fabaceae.

Lavin *et al.* (2001) placed *Pterocarpus* under subfamily Faboideae and assigned to the clade Pterocarpus, sub-tribe Pterocarpeae, within the tribe Dalbergieae. They placed legumes with samaroids pods under Pterocarpeae, pods with very small marginal wings under Lonchocarpeae and legumes with drupaceous fruits under Geoffroyeae. Previously, Polhill (1971, 1981, and 1994) also used the evidences from morphology, seed chemistry and wood anatomy and grouped the 19 woody genera from Bentham's Pterocarpeae and Geoffroyeae into new Dalbergieae.

The informal monophyletic clade Pterocarpus contains 22 genera and ca. 200 species including genus *Pterocarpus* and the members of this clade are mostly centred in the Neotropics, whereas *Pterocarpus* and *Stylosanthes* are pantropical, *Inocarpus* is Asian, and *Chapmannia* is transatlantic in terms of distribution (Lavin *et al.*, 2001).

**Holotype of Pterocarpus marsupium Roxb.**

A holotype of a species is the only specimen (or sometimes illustration) designated by the author as the "nomenclatural type". The type locality of *Pterocarpus marsupium* Roxb. is Circar mountains of the Coromandel as per the protologue (Roxburgh, 1799). The protologue of *Pterocarpus marsupium* Roxb. is based on an illustration (Figure 1).


**Figure 1:** Holotype of *Pterocarpus marsupium* Roxb. (*Source*: Roxburgh, 1799).

**Synonyms**

*Lingoum marsupium* (Roxb.) Kuntze
*Pterocarpos bilobus* G. Don
*Pterocarpos marsupium* f. *acuminata* (Prain) St.-Lag.
*Pterocarpos marsupium* f. *acuta* Prain
*Pterocarpos marsupium* f. *biloba* (Roxb. Ex G. Don) Prain
*Pterocarpos marsupium* subsp. *marsupium* Roxb.
*Pterocarpos marsupium* var. *acuminata* Prain
*Pterocarpos marsupius* (Roxb.) St.-Lag.

**Taxonomic description**

Medium to large sized deciduous tree, bark grey to dark brown coloured, often characterized by the presence of shallow cracks. Branches spreading, glabrous to pubescent. Leaves alternate, imparipinnate, leaflets 5-7, alternate, rarely subopposite, sparingly clothed beneath with persistent hairs; stipules small, caducous; petioles rounded and smooth. Inflorescence a much branched terminal panicle with yellow flowers; pedicels rusty puberulous; pedicels 0.2 cm, with two small ovate caducous, bracteoles at the apex. Calyx often incurved, obconical, 0.5-0.7 cm in length, 5-toothed; teeth short, connate. Corolla papilionaceous, exserted beyond the calyx tube, enclosing the stamens and carpel inside; petals long clawed, margin crisped; standard ovate to orbicular, waved, reflexed and veined; wings slightly twisted and reflexed; keels waved, fused at the tip. Stamens 10, united at the base and soon dividing into two bundles with 5 stamens in each resulting (5+5) arrangement towards apex; staminal sheath split open dorsally; anthers versatile, bulbous and bilobed. Ovary elliptical, pedicellate, hairy, generally bi-celled, 2–6- ovuled; style incurved, filiform, glabrous, ascending; stigma terminal. Legume indehiscent, orbicular, compressed, broadly hardened, winged around margin, borne on a long pedicel, usually 1-seeded, sometimes 2-seeded; style persistent, incurved. Seeds oblong or sub-reniform, dolabriform, hilum small. Flowering occurs during September to November and Fruiting from December to March (Figure 2 and Figure 3).

**Infraspecific taxa**

*Pterocarpus marsupium* subsp. *acuminatus* (Prain) Thoth.
*Pterocarpus marsupium* subsp. *marsupium*

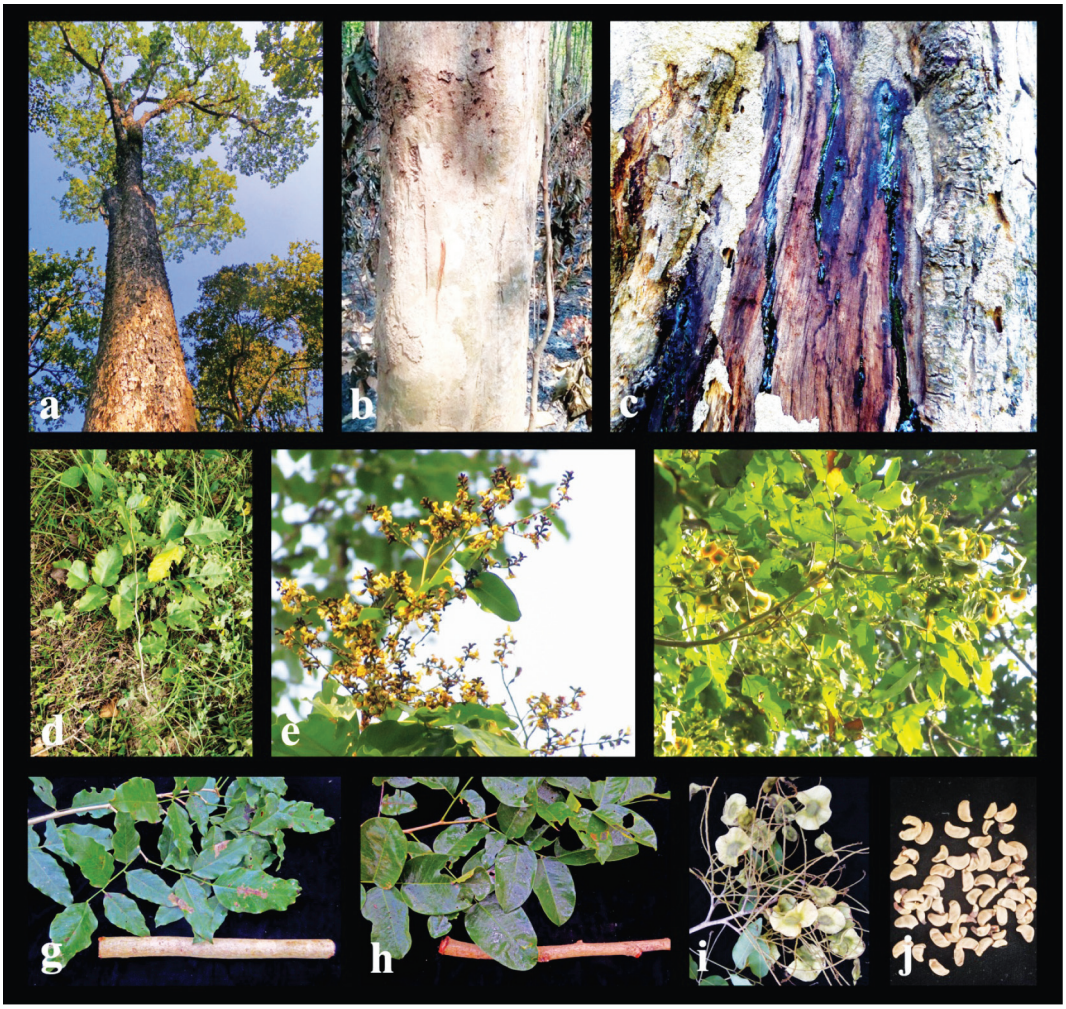


**Figure 2:** *Pterocarpus marsupium;* **a**. Tree; **b**. Trunk with termite nest during summer; **c**. Slashed bark releasing Kino gum; **d**. Seedling in wild; **e**. Flowering branch; **f**. Fruiting branch; **g**. and **h.** Variation in leaf shapes; **i.** Branch with winged fruits; and **j.** Seeds. (**Photos:** a., c., g., and h. Ganga Datta Bhatt; b. Ghanashyam Chalise; d. Pratikshya Chalise; e. and f. Neelam Pandey; i. Amrit KC; and j. Ram Krishna Bhandari).

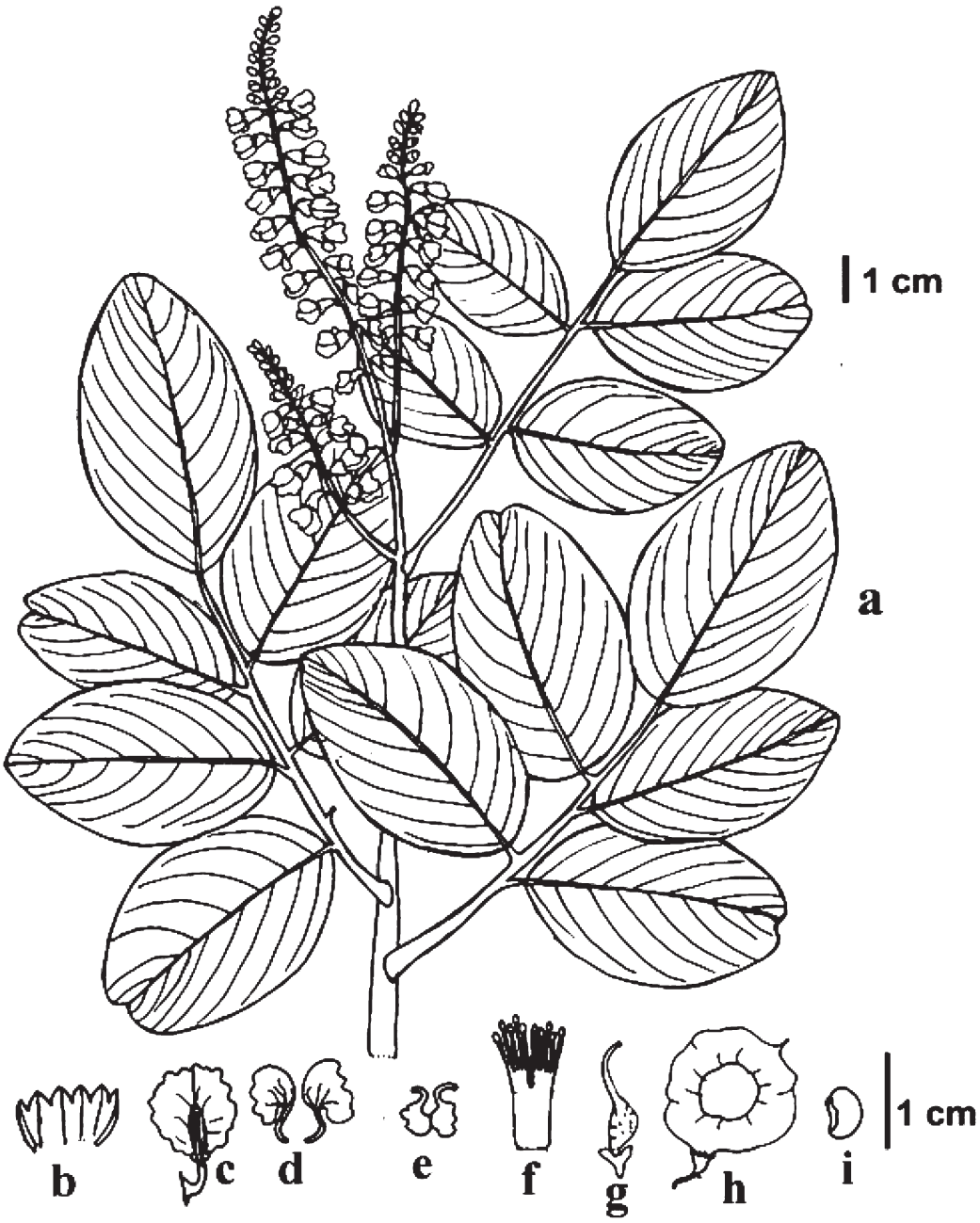


**Figure 3:** Illustration *Pterocarpus marsupium* Roxb.: **a**. Habit; **b.** Calyx tube (opened); **c.** Standard petal; **d.** Wings petals; **e.** Keels; **f**. Staminal tube opened; **g**. Carpel; **h.** Pod; and **i**. Seed. (KATH070868, Kurmi, P.P. 10084 and KATH070855, Bhatt, G.D. and Kurmi, P.P. 1006. **Illustration:** drawn by Pratikshya Chalise).

# CHAPTER - 3
# REPRODUCTIVE BIOLOGY

**Pratikshya Chalise**

Reproductive biology broadly incorporates floral biology, phenology, pollination mechanism, fruit and seed ontogeny of plants. These aspects must be clearly understood to carry out a quality research on breeding programme and efficiently manage seed orchards or maximize seed production (Pulliah, 2019(a)). Together, the inflorescence structure also determines the spatiotemporal arrangement of the flowers during anthesis and is therefore crucial for reproductive success in plants (Prenner, 2013).

In *Pterocarpus marsupium*, the flowers are insect pollinated especially by honey bees. Studies have shown that the species with yellow flowers are mostly pollinated by different species of bees, as bees prefer yellow flowers (Endress, 1995). However, successful pollination not only depends on the pollinator but it is also determined by the flower-opening time and duration, anther dehiscence, stigma receptivity and germination of pollen tubes.

Very little studies have been carried out on the sexuality as well as pollination mechanism of this plant which indicates that *P. marsupium* exhibits mixed breeding system with both self- pollination and cross-pollination (Pal and Mondal, 2018), but both self- and cross-pollinations depend on pollen mediation by the flower visitors (Pulliah, 2019(a)). However, Rao *et al.* (2001) also indicated that there is large-scale abortion of flower buds, flowers and fruits. Being a multipurpose tree species, Bijaysal should have been a matter of concern to researchers, ecologists, forest scientists, environmentalists and policy makers but the reproductive biology of the plant is poorly understood. Restricted distribution patterns, low germination rate, prolonged dormancy period, slow growth, long juvenile period and tough access to mature flowering trees may be reasons for its limited study (DoF, 2018; Pulliah, 2019(a)).

## Materials and Methods

Present investigation was carried out based on the observations in Bijaysal trees growing in different parts of the country from October 2019 to February 2021. The records of herbarium specimens housed at KATH as well as relevant secondary sources were also referred to draw the inferences. Similarly, to study the floral structure as well as for palynological examination; flower samples were collected in 30% alcohol from Kailali district, Western Nepal during November 2020. Similarly, to study the morphological structure of fruits and seeds, pods of Bijaysal were collected from Nepalgunj during January, 2021.

Floral structures as well as palynological studies were carried out in National Herbarium and Plant Laboratories (KATH), Godawari. Flower samples were rehydrated in distilled water mixed with a drop of detergent (Rajbhandary, 2015) and observed under stereomicroscope and individual floral parts were photographed using Nikon Coolpix 2800 camera. For the study of pollen grains, the anthers were taken by a dissecting needle, placed in glass slides, teased anthers were stained with 1% safraine solution, covered with cover slips and observed under the microscope and measured using calibrated ocular. Pollen shape was determined based on the values of P/E ratio as documented by Erdtman (1952).

Pollen viability analysis was carried out by following Iodine-Potassium Iodide (IKI) and Tetrazolium test (TTC test). The total number of stained pollen grains per microscopic field was counted for twenty observations and pollen viability (%) was calculated using the following formula (Hauser and Morrison, 1964);

$$\text{Pollen Viability (\%)} = \frac{\text{No. of stained pollen grains}}{\text{Total no. of pollen grains per microscopic field}} \text{ X } 100$$

However, information on the pollination biology of Bijaysal was also gathered from secondary sources.

**Phenology**

Bijaysal exhibits leaves fall by the end of February and undergoes a brief deciduous phase between April to May and new foliage starts to appear from May to June. Similar observations were made by Pal and Mondal (2018) from West Bengal, India. Bijaysal is a mass blooming legume tree and flowering starts during August and lasts up to November whereas fruiting during December to March.

**Inflorescence structure and flower opening**

The flowers in Bijaysal are minute ca. 1.6 cm, bright yellow coloured and are borne in branched, terminal panicles. Pal and Mondal (2018) reported that these minute flowers open at 05.30 h and continued upto 06:30 h during which they emit mild fragrance as well as nectar.

**Flower morphology and structure**

The flowers are bright yellow, 16 mm long, typically papilionaceous, zygomorphic, bisexual and pedicellate, and with mild fragrance. Sepals- five, green, joined at the base to form a narrow tube, ca. 0.6 cm x 0.3 cm, slightly bent forming curvature, persistent (Figure 1a,b). Petals-5, vexillary aestivation, yellow, standard petal posterior, ca. 13 mm in length, long clawed, ovate with crisped margins, veins

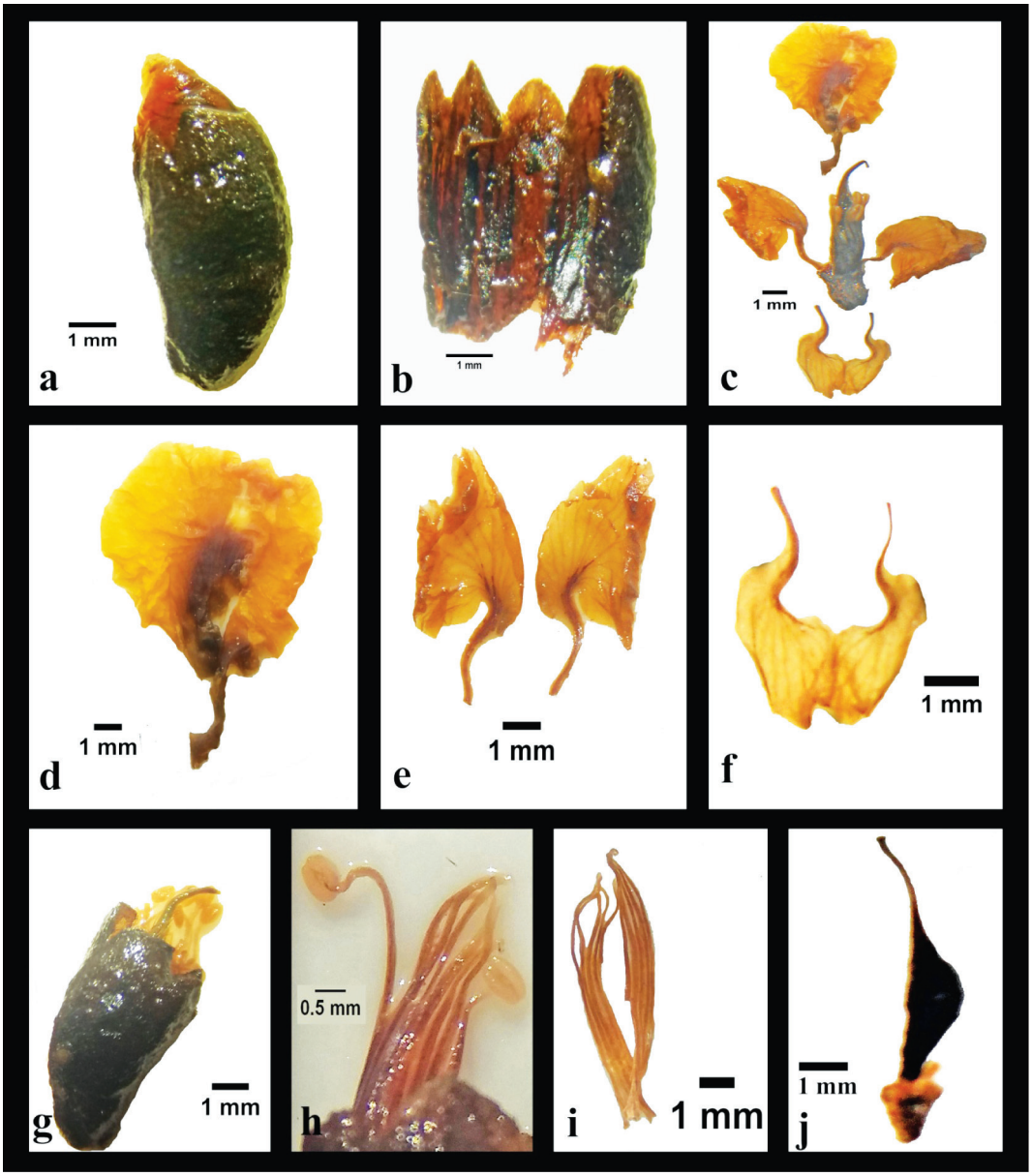


**Figure 1:** Floral anatomy of *Pterocarpus marsupium* Roxb. (different floral parts observed under stereomicroscope); **a.** Bud; **b.** Calyx tube dissected; **c.** Dissected flower; **d.** Standard petal; **e.** Wing petals; **f.** Keels; **g.** Unequal stamens extending out of calyx tube; **h.** Stamens with versatile anther; **i.** Stamens with 5+ 5 arrangement opened at the base; and **j.** Pedicellate carpel with capitate stigma, slender style and ovary. All the structures photographed under stereomicroscope. (**Photos**: Pratikshya Chalise).

prominent, brownish; lateral two winged petals, ca. 7 mm in length, clawed, ovate with crisped margins, veins prominent, brownish; the two keel petals, ca. 4.5 cm in length, fused anteriorly, free at the base, margin curved, veined (Figure 1c,d,e,f). Both androecium and gynoecium are concealed within the keels. Stamens unequal in length, diadelphous, with ten anthers arranged in two bundles of five anthers each

(Figure 1g,h,i). Such 5+5 arrangement of stamens was also reported by Rao and Raju (2002). Each stamen coherent with the adjacent stamen on both sides, except for the extreme ones. Filaments are fused upto more than half of the length from the base, forming a more or less tubular structure; anthers are dithecous, bulbous, light yellowish to creamy coloured, versatile. Carpel ca. 0.9 cm, pedicelled, oblong, slightly bent; ovary hairy, unilocular, superior ovary with two ovules; style ascending, glabrous, stigma capitate (Figure 1j). Sinjushin (2019) also reported pentamerous and pentacyclic flower with a monomerous gynoecium (K5 C5 A5+5 G1).

During bud stage, the standard petal encloses the wing and keel petals which in turn enclose both the sex organs (Figure 1a). As the bud matures, the standard petal gradually bulges out and protrudes outwards from the calyx tube. Both the style and the staminal tube follow the keel's curvature upwards, and thus appear slightly bent; both anthers and stigma are positioned around the keel apex. Similar structure was reported by Pinheiro *et al.* (2018) and Pulliah (2019(a)) in various species of *Pterocarpus*.

Nectar is accumulated around the base of the gynoecium and is confined within the chamber formed by the fusion of the filaments. Thus, it can be reached only by small openings on the base of the upper side of the staminal tube, whose access is blocked by the claw of the standard petal (Pinheiro *et al.,* 2018). These specialized structures make the flowers of Bijaysal mechanically isolated such that they require specific pollinators for pollinating these complex flowers. Pal and Mondal (2018) reported that flower visitors in Bijaysal include the members of Hymenoptera, Lepidoptera and Thysanoptera where bees were found to be the most dominant and the most effective one. These flower visitors are rewarded with both nectar and pollens. According to Pal and Mondal (2018), during the time of flower opening nectar is absent but the secretion of nectar increases gradually with time.

**Anthesis and Palynological observation**

In Bijaysal, the standard petal encloses the wings and keels which in turn enclose both the reproductive structures, thus anther dehiscence can take place only after the unfolding of these petals. Anthers are tetrasporangiate and dehiscence of anthers takes place by the formation of longitudinal slits (Figure 2a). According to Pulliah (2019(a)), dehiscence of anther takes place asynchronously; two anthers an hour after anthesis, three anthers again after one hour and the remaining five again after 1.5 hours.

Pollen grains are medium sized, 19.64 - 22.58 µm, spheroidal, psilate, tricolporate with rounded poles. Grain arrangement was found to be monad (Figure 2c,d,e). Panicker (2004) considered monads are as the simplest arrangement in the evolutionary line and polyads as the most advanced type of grain arrangement. Interestingly in-vivo

germination of pollen grains inside the anther was also observed, and the pollen tubes were of variable length in different pollen grains. The TTC test for pollen viability, viable pollens stained red while the non-viable did not take stain. The stained pollen grains were counted to calculate the pollen viability which was found to be 80% (Figure 2b). However, in IKI test, pollen did not take the stain. The result is in accordance with Pal and Mondal (2018), which reported 82% pollen viability using TTC test. Studies have shown that each anther produces approx. 3,518±12.79 pollen grains and a single matured flower produces approx. 35,180±127.95 pollen grains (Pal and Mondal, 2018).

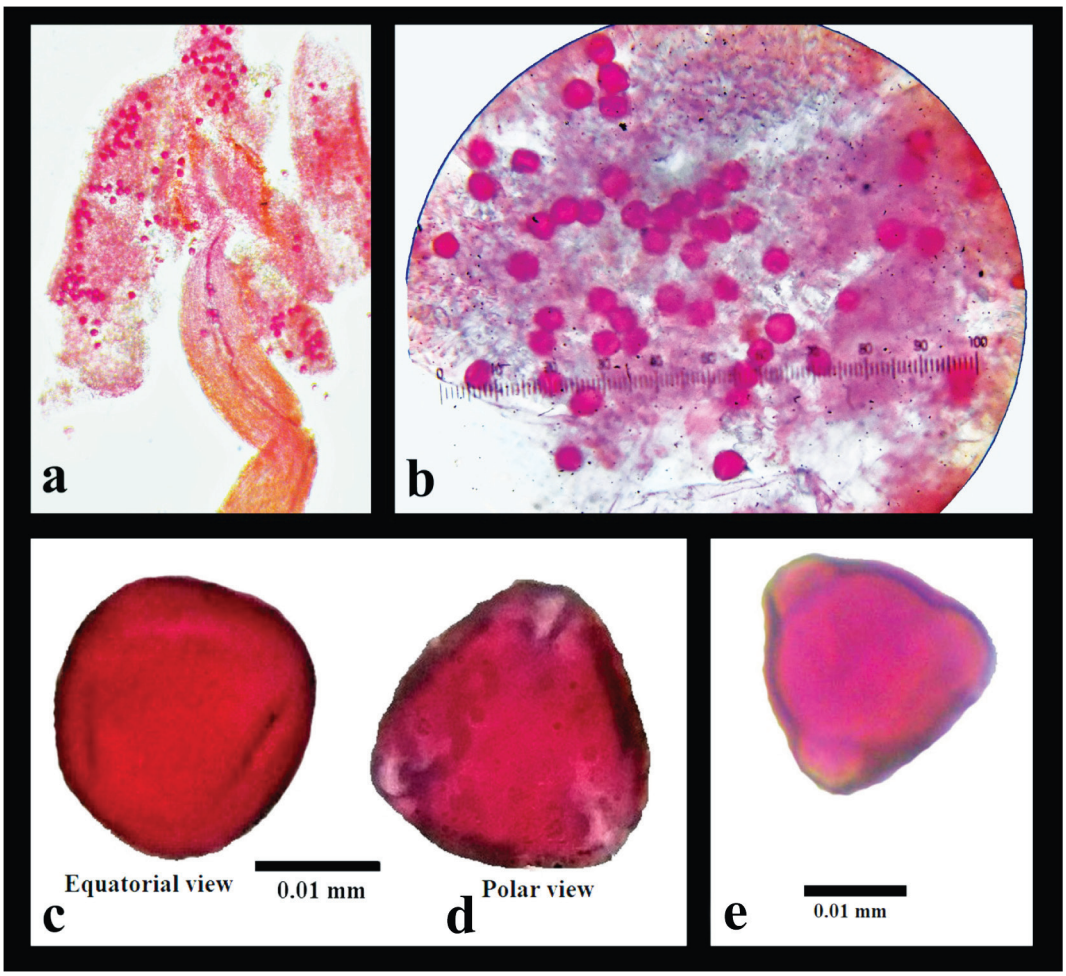


**Figure 2:** Palynological observation in *Pterocarpus marsupium* under Olympus E 329153 microscope; **a.** Longitudinal dehiscence of anther showing pollen grains; **b.** Pollen viability test (v- viable pollen; nv- non viable pollen); **c.** Single, tricolporate pollen grain (equatorial view); **d.** Tricolporate pollen (polar view with three colpi); and **e.** Tricolporate pollen showing rounded poles (polar view with three poles). Magnification: a. 100X, b., c., d., e. 400X. (**Photos:** Pratikshya Chalise).

## Pollination biology

In *Pterocarpus*, the stigma becomes receptive by about the time the first two anthers dehisce and remains so until late evening of the same day (Rao and Raju, 2002). Since, the flowers are zygomorphic and have semi-closed petals, they are mechanically strong with good landing platform (Pulliah, 2019(a)).

Bees land on the corolla wing-keel complex and direct their heads towards basal portion of the staminal tube where they extend the proboscis through small openings to reach the nectar (Pulliah, 2019(a)). This causes the flower keel to move down, exposing anthers and stigma; which then touch the ventral side of the bee's abdomen such that pollen grains get attached on their underside and are transferred to other conspecific stigmas during their subsequent visits. After the bees depart, the petals would return to their original position. But sometimes the flowers may remain unvisited for several days or even weeks because of the absence of pollinator activity, especially during dry and hot conditions.

## Morphology and Ontogeny of Fruits and Seeds

Fruits orbicular, ca. 6.2 cm long and 4.5 cm wide; winged, wing more or less rounded; pedicellate; with persistent calyx and style even at the maturity of fruit (Figure 3b). Style more distinct in young stage (Figure 3a). Seeds usually one, sometimes two, mean size 5x3 mm, brownish, slightly kidney shaped, dolabriform and glabrous (Figure 3c).

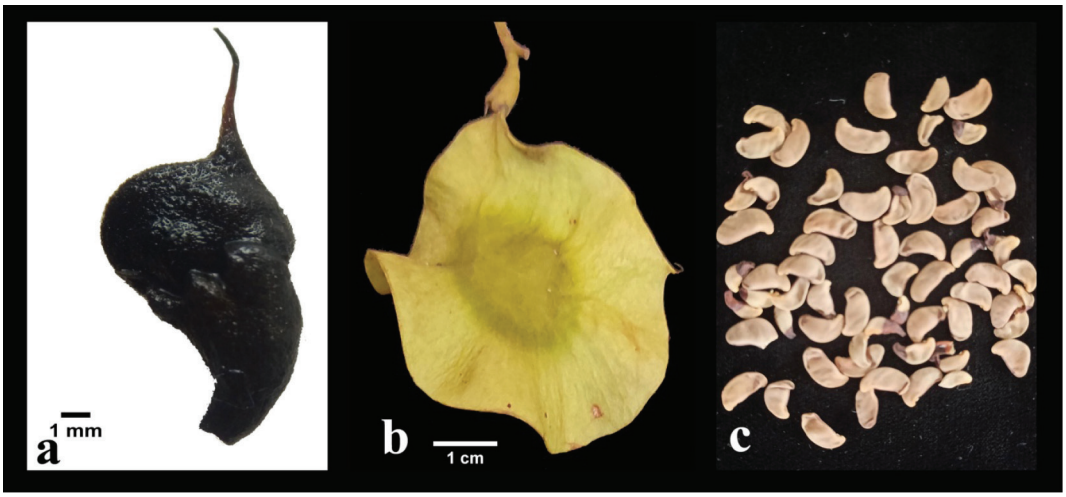


**Figure 3:** Morphology of fruit and Seed in *Pterocarpus marsupium*; **a.** Young fruit; **b.** Matured fruit and; **c.** Seeds. (**Photos:** a. Pratikshya Chalise; b. Amrit KC; and c. Ram Krishna Bhandari).

*Pterocarpus* is able to fruit through both self- and cross-pollination so the fruit production is high (Pal and Mondal, 2018). But, the offspring especially those resulting from self-pollination may either become inviable or, become sterile and these poor offspring are gradually and selectively eliminated from the population. Thus, despite of very high flower production, the natural fruit set is very low (Pulliah, 2019(a)).

# CHAPTER - 4
# ANATOMY

**Pratikshya Chalise and Lajmina Joshi**

Several studies have been done on phytochemistry, pharmacognosy of *Pterocarpus marsupium* but only few scientists (Rao and Juneja, 1971; Gamble, 1972; Pearson and Brown, 1932) have done the anatomical studies of the wood. Metcalfe and Chalk (1957, 1989) has also mentioned the anatomy of wood, stem, bark, leaf and petiole in the members of several angiosperm families in his book "*Anatomy of Dicotyledons*". Chauhan and Rao (2003) has also mentioned about the anatomical features of the legumes including genus *Pterocarpus* in their book "*Wood Anatomy of Legumes of India: Their Identification and Uses*" but detailed study on Bijaysal has not been done until now.

In present scenario, vigorous increase in global trade has resulted in over-exploitation of forest resources imposing serious threats over the biodiversity. Therefore, developing accurate species level identification through their anatomical studies is a crucial and significant technical prerequisite. In Nepal, anatomical studies have not been done so far regarding this species. The present study has thus been undertaken to highlight the anatomy of different parts of *P. marsupium* such as wood, leaf, bark, petiole, rachis and petiolule in order to give detail information about their anatomical characteristics and to help in identification of the Kino tree.

## Materials and Methods

For anatomical studies the samples of wood and leaves were collected from Rupandehi in November, 2019 and Kailali and Kanchanpur districts in April, 2020. For the wood sample, a small cubic block (2-3 cm in side length) of wood was taken from the outermost part of the tree (GoN, 2012). The blocks, leaves, bark and petiole were fixed and preserved in FAA (Formalin- Acetic acid- Alcohol). Before sectioning, wood blocks were boiled in order to soften so that section can be taken easily. After softening, thin sections (20-40 μm) of transverse, longitudinal and radial faces were cut using a sliding microtome. The sections were stained in Safranin and Fast green; dehydrated in alcohol series. Permanent slides were prepared by mounting in DPX mountant. Maceration of wood chips was done using Jeffrey's solution and the temporary slides were prepared to observe the detailed structure of vessel, phloem, fibres, etc. Similarly, transverse sections of petiole, rachis, petiolule and leaves were also taken and permanent slides were prepared. Stomatal study was done by peeling the epidermal layer, which was done by boiling small pieces of leaves in Jefferey's solution at 60ºC in hot air oven for softening. Epidermal peels were washed thoroughly, stained in aqueous Safranin. Temporary slides were prepared

mounting in diluted glycerine and then sealed by nail polish. The permanent as well as temporary slides were then studied under compound microscopes at different magnification and photographs were taken.

Stomatal Index (SI) and Stomatal Frequency (SF) were calculated using the formulas (Rajbhandary, 2015).

Stomatal Index (SI)= S X 100/ (E+S)

where, S= average no. of stomata in microscopic field

E= average no. of epidermal cells in microscopic field

Stomatal Frequency (SF)= S/A per $mm^2$

where, S= average no. of stomata in microscopic field

A= area of microscopic field

**Anatomy of Stem**

The transverse section of stem is ribbed due to the presence of fissured bark. The epidermis consists of a single row of rectangular cells that is covered with cuticle. In young stem, some of the epidermal cells give rise to multicellular hairs. Epidermis is followed by collenchymatous hypodermis which consists of three to four layers of cells. Below hypodermis, multilayered cortex is present that consists of thin walled, parenchymatous cells. Endodermis is single layered and is made up of elongated, barrel shaped cells. The endodermal region is followed by pericycle that consists of sclerenchymatous cells. The stele consists of conjoint, collateral and open vascular bundles arranged in ring. The bundles are relatively different in size and number. There are six large bundles located opposite ridges. A large pith is present in the centre of stem which consists of polygonal parenchymatous cells that tend to decrease in size towards the periphery. However, in matured stem, due to the presence of periderm, the epidermal cells as well as hypodermal region seem to be rudimentary.

**Wood anatomy**

Wood very hard, close grained, somewhat heavy and durable. Heartwood yellowish brown to brown, that gradually turns brown with age. Heartwood fluorescent, staining yellow when damp and gives brownish colour in aqueous solution. The colour of this aqueous solution becomes faint after frequent use. Sap wood pale yellowish to yellowish-white, narrow, distinct from the heartwood. Growth rings not very distinct. Pores usually minute, visible only under the lens but sometimes large and clearly visible to the eyes.

Wood is diffuse porous type. Vessels few, 7-10 vessels per $mm^2$, mostly solitary, sometimes in short radial multiples of 2-4 (Figure 1a,b). Solitary vessels oval in outline; 90-251 (165) µm and 74-177 (165) µm in radial and tangential diameter respectively (Figure 1a,b). Vessel elements 62-79 (70) µm long, end wall oblique. Perforation plates simple. Inter- vascular pits alternate, bordered, 4.8-5.2 (5) µm in diameter (Figure 1i), but two distinct diameter classes of vessels are absent. According to the Inside Wood Database (http://insidewood.lib.ncsu.edu, retrieved on 02-03-2021), the vessels in *P. marsupium* range between 100-200 µm in size and the number ranges between 5-20 vessels per $mm^2$. However, Chalk (1989) considers the size as well as number of vessels susceptible to environmental influence. Anoop *et al.* (2019) also reported that vessel diameter as well as vessel element length both decrease whereas the number of vessels increases with increase in aridity. Heartwood vessels sometimes filled with gums and other deposits (Figure 1b), but tyloses are absent. Chauhan and Rao (2003) reported the occurrence of brown or orange- brown gummy deposits in vessels.

Fibre tracheids constitute the ground mass of the wood, square to polygonal in outline; 7-19 (12.8) µm in diameter, thick walled, 5-8 (6) µm; 520-780 (750) µm long, non-septate (Figure 1i). Pits simple, small, round with slit like apertures. Fibres are often attached with the ray parenchyma. Fibres greatly influence the strength as well as shrinkage of the woods (Anoop *et al.*, 2019).

Wood parenchyma paratracheal, vasicentric; aliform confluent; arranged in narrow tangential bands, square, round or polygonal in outline; 12-36 (24) µm and 9-35 (18) µm in radial and tangential diameter respectively; thin walled (Figure 1b); 1-2 celled, septa nodular (Figure 1e); few storied (Figure 1c). Vessel parenchyma pit similar to vessel pit, oval, bordered, alternate, 5-10 (8) µm in horizontal diameter (Figure 1h). Crystals present (Figure 1e). Metcalfe and Chalk (1989) also reported the presence of solitary crystal in majority of the members of family Fabaceae. Chalk (1989) reported the aliform confluent parenchyma as the most advanced type of parenchyma.

Rays homogenous, 1-3 cells wide, made up of parenchymatous tissue; mostly uniseriate, few multiseriate; very fine and distinct (Figure 1c,d,e), composed of procumbent cells only. Uniseriate rays 4-5 celled, 85- 137 (104) µm in height (Figure 1d); multiseriate rays 2-3 celled, 14-31 (23) µm in width and 109-177 (134) µm in height (Figure 1e). Gamble (1972) described these rays as fine, white, wavy, often interrupted concentric lines visible in the cross-section of wood. Procumbent cells oval, vertically elongated in tangential section, 60-100 (82) µm and 17-25 (20.5) µm in radial and tangential diameter respectively (Figure 1d,e,g). Ray vessel pit 3.24- 5.91 µm (4.41µm) in diameter (Figure 1h). Crystals absent.

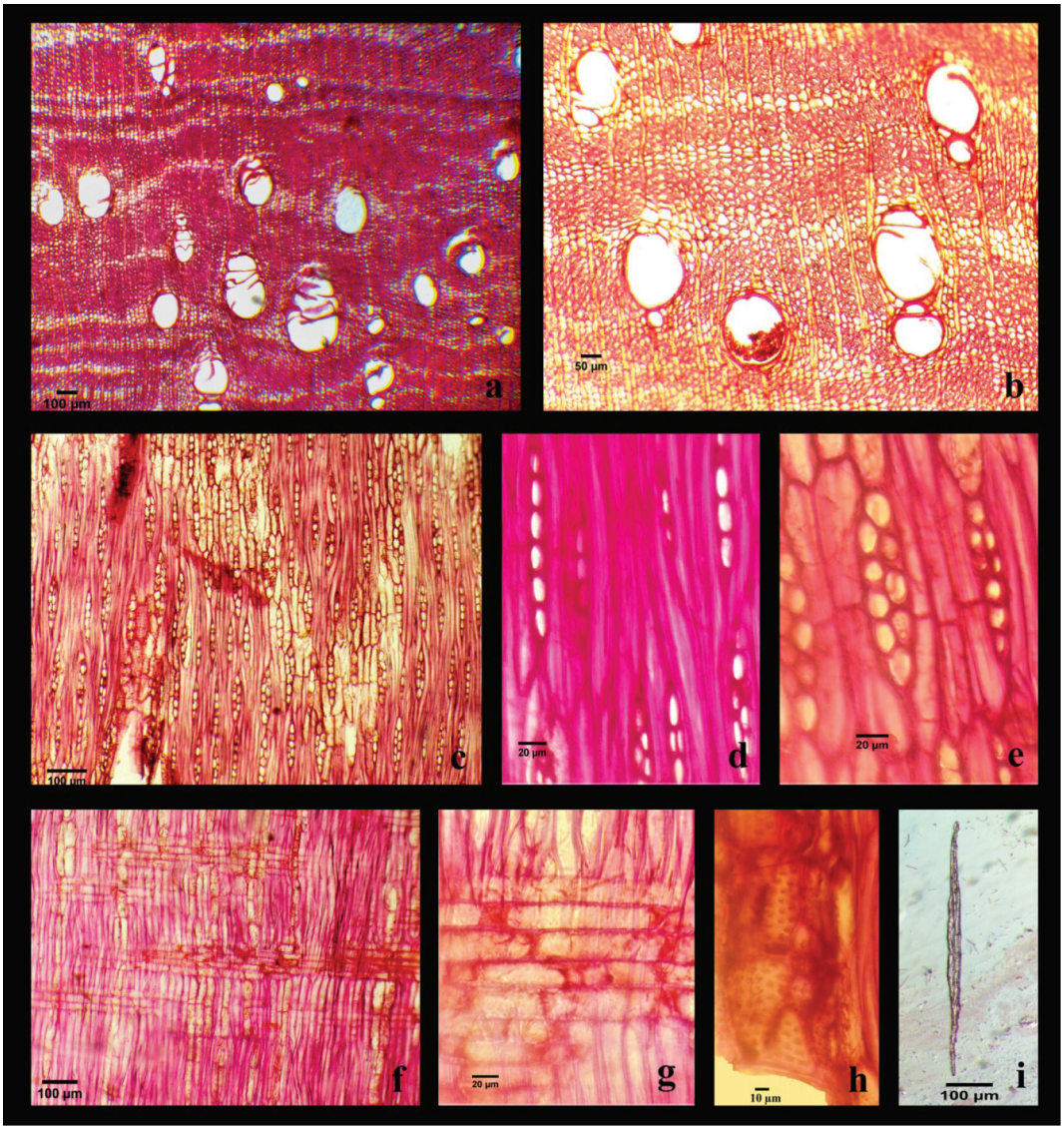


**Figure 1:** Wood anatomy of *Pterocarpus marsupium* **a, b.** Cross-section of wood (TS) showing solitary as well as short radial multiple of vessels, paratracheal, aliform confluent arrangement of parenchyma, vessels with deposits; **c. d. e. h.** TLS of wood showing vessels, fibres and few stories parenchyma in (c); uniseriate rays in (d); multiseriate rays and two celled parenchyma in (e); **f. g.** RLS of wood showing homogenous rays and crystals in parenchyma in (f); and procumbent cells with pitted vertical and horizontal wall in (g); and **i.** Fibres in macerated wood samples. Magnification- a. (4X+ 0.5X), b., c., f. (10X+ 0.5X), d., e., g., h., and i. (40X+ 0.5X) (**Photos**: Pratikshya Chalise).

## Anatomy of Petiole and Petiolule

The transverse section of petiole shows the following features. The epidermal region is uniseriate, glabrous, with rectangular shaped cells and is covered with thick cuticle. Collenchymatous hypodermis is located under the epidermis. The cortex consists of orbicular parenchymatous cells. Cortical region consists of few darkly stained secretory cells (Figure 2a). Endodermis is single layered, slightly wavy in outline and is made up of slightly elongated parenchymatous cells. It is followed by patches of sclerenchymatous pericycle (Figure 2b). Heneidak *et al.* (2008) also suggested the presence of pericycle fibres in family Fabaceae including genus *Pterocarpus*. Between sclerenchymatous patches some cells are enlarged. Vascular strands conjoint, collateral and open type; with radially elongated xylem elements towards the inner side and phloem towards the outer side. Cambium is present between xylem and phloem. The centre of the petiole is occupied by parenchymatous pith. Tanniniferous canals present in the pith just below the xylem elements (Figure 2b). Darkly stained deposits present in cortex and phloem.

The transverse section of rachis shows the following features. Epidermis is single layered, glabrous, with thick deposition of cuticle. Hypodermis is absent. Cortex is highly reduced compared to petiole (Figure 2c). Cortex 2-3 layered, parenchymatous. Endodermis is single layered; wavy in outline; made up of slightly elongated parenchymatous cells. Pericycle is in sclerenchymatous patches. Vascular strands conjoint, collateral and open type with radially elongated xylem elements towards the inner side and phloem towards the outer side. Cambium is present between xylem and phloem. The center of the petiole is occupied by parenchymatous pith. Tanniniferous canals present in pith just below xylem elements (Figure 2c). Darkly stained deposits present in cortex and phloem.

Anatomically petiolule is similar to the petiole. Unlike petiole, the transverse section of petiolule shows wavy outline and is covered with dense trichomes.

## Anatomy of Leaves

Leaves in *P. marsupium* are dorsiventrally flattened, hypostomatic due to the presence of stomata on the lower epidermis, with prominent midrib (Figure 2d,e). Upper epidermis (adaxial surface) is dark green in colour compared to lower epidermis (abaxial surface). The upper epidermis is single layered, with square shaped cells, thick walled, covered with thick cuticle (Figure 2e); anticlinal walls straight (Figure 2f). Lower epidermal cells oval, angular in outline; thick walled; undulate or slightly sinuous anticlinal walls (Figure 2e,g,h). Stomata and trichomes present in lower epidermis only.

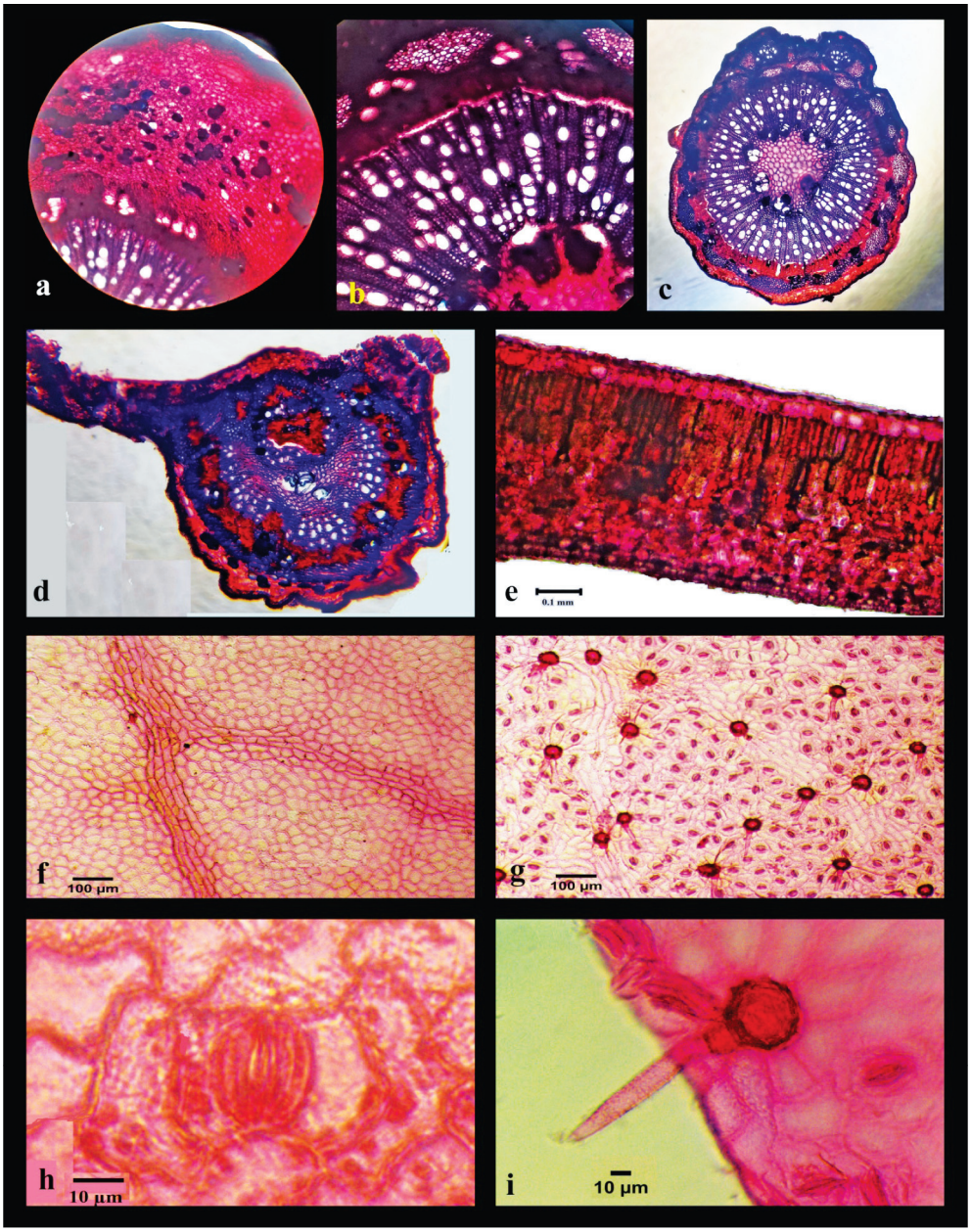


**Figure 2:** Anatomy of petiole, rachis and leaf; **a. b.** TS of Petiole showing cortical region with tanniniferous cells in (a); Stellar region with phloem and xylem, Tanniniferous canal in pith in (b); **c.** TS of rachis showing sclerenchymatous pericycle, vascular strand, tanniferous canal in pith; **d.** VS of leaf through mid-rib, showing single large C-shaped vascular bundle and a small vascular bundle, both surrounded by sclerenchymatous bundle sheath; **e.** VS of leaf through leaf lamina, showing palisade and spongy parenchyma; **f.** Upper epidermis with smooth anticlinal walls; **g.** Lower epidermis with stomata and trichomes; **h.** Paracytic stomata with two subsidiary cells parallel to the guard cells, epidermal cells with undulate anticlinal walls; and **i.** Unicellular, glandular trichome in lower epidermis. Magnification: a, b (100X), c, d, e, f, g (10x+0.5X), h, i (40X+0.5X) (**Photos:** Pratikshya Chalise).

Stomata paracytic (Rubiaceous type). Each stoma is surrounded by two subsidiary cells that lie parallel to the guard cells (Figure 2h). Guard cells typically kidney-shaped and ostiole are located on the same level relative to the epidermal cells. Stomatal length ranges from 20-27 (22.4) µm. Stomatal frequency 223.2 per $mm^2$ and Stomatal index 40.21. Idu *et al.* (2006) also reported hypostomatic stomata as the characteristic feature of family Fabaceae. They reported the occurrence of anomocytic stomata with agenous ontogenic pathway as primitive character and the occurrence of paracytic stomata with eumesogenous ontogenic pathway as advanced characteristics within Fabaceae.

Trichomes unicellular, glandular; 96-188 (129) µm long, 18 per $mm^2$; basal glands 28-38 (32) µm in diameter (Figure 2i). Trichomes are more clustered and shorter in the region of veins, whereas longer and distantly present in the other part of leaf blades compared to veins.

The mesophyll lies just beneath the epidermis; dorsiventral, heterogeneous; differentiated into palisade and spongy parenchyma (Figure 2e). Palisade parenchyma 2-3 layered; made up of elongated cells; vertically arranged; parallel to each other. Palisade zone does not exceed the upper epidermis of midrib. Spongy parenchyma lies below palisade parenchyma; made of up round to oval cells; loosely arranged, which extend upto the lower epidermis.

Midrib is externally bound by single layered epidermis. Mesophyll tissue is absent in the midrib. Epidermis is followed by collenchymatous hypodermis. Cortex consists of orbicular parenchymatous cells. Vascular system comprises of one large 'C'-shaped vascular bundle and one smaller vascular bundle lying opposite to the large bundle (Figure 2d). Vascular bundles are conjoint, collateral and closed type with varied shape and size from center to margin. Each vascular bundle consists of closely arranged, radial files of narrow angular xylem elements towards the inner side and phloem towards the outer side (Figure 2d). The cortical vascular bundles are smaller in size; conjoint, collateral and closed type. At the center lies the parenchymatous pith. Darkly stained deposits found in phloem and pith.

# CHAPTER - 5
# DISTRIBUTION

**Yagya Raj Paneru, Pratikshya Chalise and Mohan Dev Joshi**

*Pterocarpus marsupium* Roxb. (Bijaysal), is an important tree species occurring in the lowlands of Nepal. Its distribution is restricted in patches and the natural stands of the tree are disappearing rapidly (Anis *et al*., 2005). Bijaysal is confined within an altitudinal range of 100 to 1200 m, and has a limited population in both global as well as national level (Barstow, 2017; DoF, 2018; Sukhadiya *et al.*, 2019). Global distribution of Bijaysal is also limited as it extends across the Indian Peninsula and northwards up to the foot of the central Himalayas (Troup, 1921). In Nepal, this species is naturally distributed at the foothills of Siwalik in Kanchanpur, Kailali, Bardia, Banke, Kapilvastu, Rupandehi and Nawalparasi (now Nawalpur and Parasi) districts (Jha, 1999; DoF, 2018) and a few trees have also been traced in Palpa and Arghakhachi districts as well (DoF, 2018). Records of herbarium specimens housed at KATH also shows that natural populations of Bijaysal occur in the lowlands of Western and Central Nepal. Similarly, in Eastern Nepal the species exists in planted condition (Rajbhandari *et al.*, 2020).

The IUCN Red List of Threatened species has listed Bijaysal in 'Near Threatened' category (Barstow, 2017). Similarly, the GoN has also prioritized this species for its conservation and for sustaining its population in long run, the first species level conservation action plan for plant species was developed in 2018 as "*Bijaysal Conservation Action Plan 2018-2022*" (DoF, 2018). However, these attempts might have often failed to be supported or have simply been super-shaded due to lack of detailed information on their biology, distributions, level of trade, population status and associated range reductions. Therefore, this chapter describes distribution and the factors governing patchy distribution of Bijaysal in Nepal.

## Materials and Methods

Present study was carried out to predict the suitable habitat of Bijaysal in Nepal using Ecological niche modeling. A total of 30 presence points were obtained from the herbarium records as well as from field observation. Similarly, 19 bioclimatic variables were considered, out of which few significant variables were selected considering correlation among the variables and biological significance for the species. Modelling was done within the distribution range of the *P. marsupium* across the tarai region of Nepal (Figure 1).

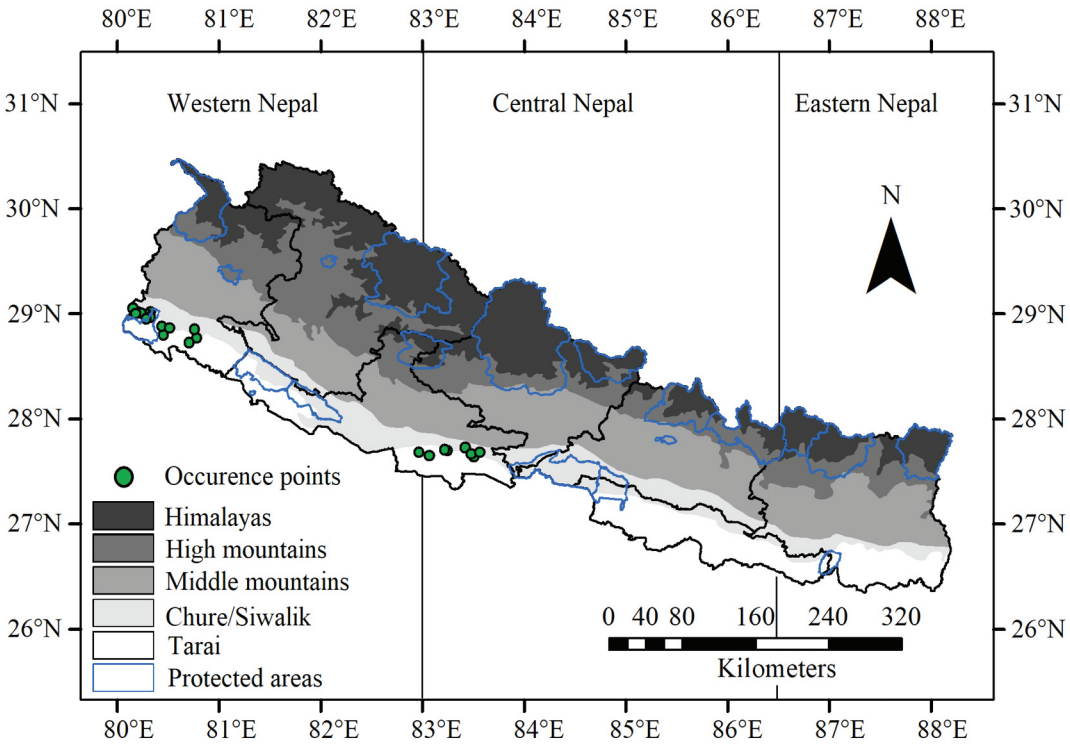


**Figure 1:** Map of Nepal showing the occurrence points of *Pterocarpus marsupium* in Nepal.

## Potential distribution in Nepal

The field-based observation, herbarium data as well as personal communication showed the distribution of Bijaysal in Central and Western Nepal from Parasi to Kanchanpur districts. The study indicates the foothills of Siwalik and Tarai of Central and Western Nepal as the potential area for distribution of *P. marsupium* (Figure 2). High habitat suitability was found in Western Nepal as compared to Central Nepal. However, in Eastern Nepal, habitat was found to be unsuitable for Bijaysal. The four districts viz; Banke, Bardiya, Kailali and Kanchanpur of Western Nepal occupy most of the probable potential area and also these districts consist of highly suitable areas. Dang district shows very low potential area. Similarly, three districts, namely Parsa, Bara and Rautahat also showed some potential area, but there is no record of the presence of this plant in natural habitat in these areas. Presence points from Banke and Bardiya were not found as most of the potential area lies within the two protected areas; Banke and Bardiya National Parks.

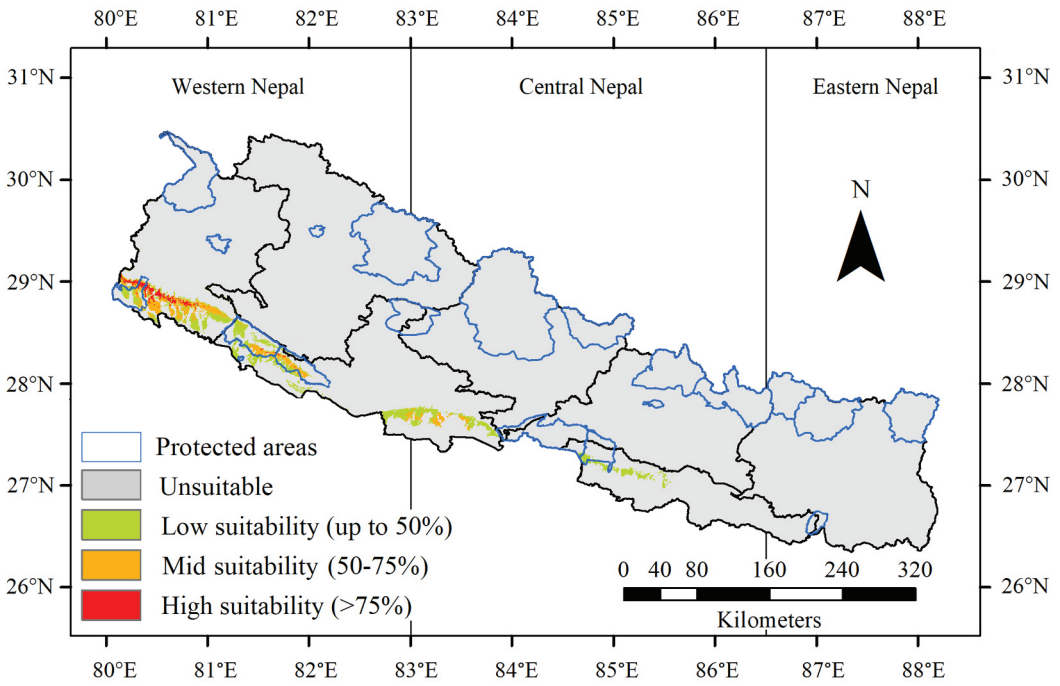


**Figure 2:** Current potential habitat of *Pterocarpus marsupium* in Nepal based on MaxEnt model.

## Factors governing Current distribution

Environmental factors play an important role in predicting the distribution of *P. marsupium*. The jackknife test revealed that the variable with highest gain when used in isolation was Soil type (Figure 3). Soil type also decreased the gain when it was omitted from the analysis, thus it has importance in determining the potential

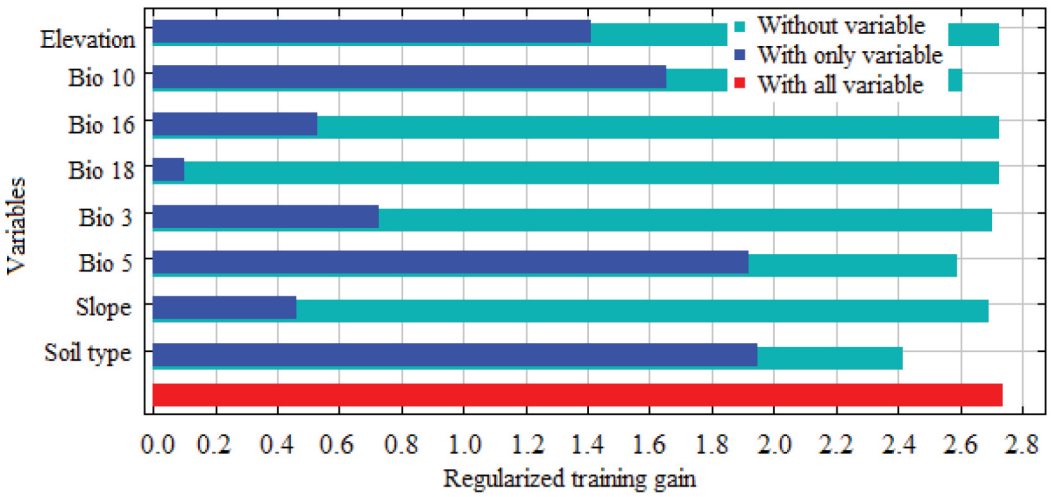


**Figure 3:** Average regularized training gain for each variable for 50 replicates (Jacknife test result).

habitat. Calcaric phaeozems soil with slope of 13°-18° were favorable for Bijaysal. Precipitation of wettest quarter (Bio 16) and warmest quarter peaks around 2326.43 mm and 131.37 mm respectively. Most probable area for suitability were within 38.86° maximum temperature of warmest month and 28.14° mean temperature of warmest quarter. This result from Jacknife test also justifies high suitability of the studied species in Western Nepal since the temperature in Western Nepal falls within this range.

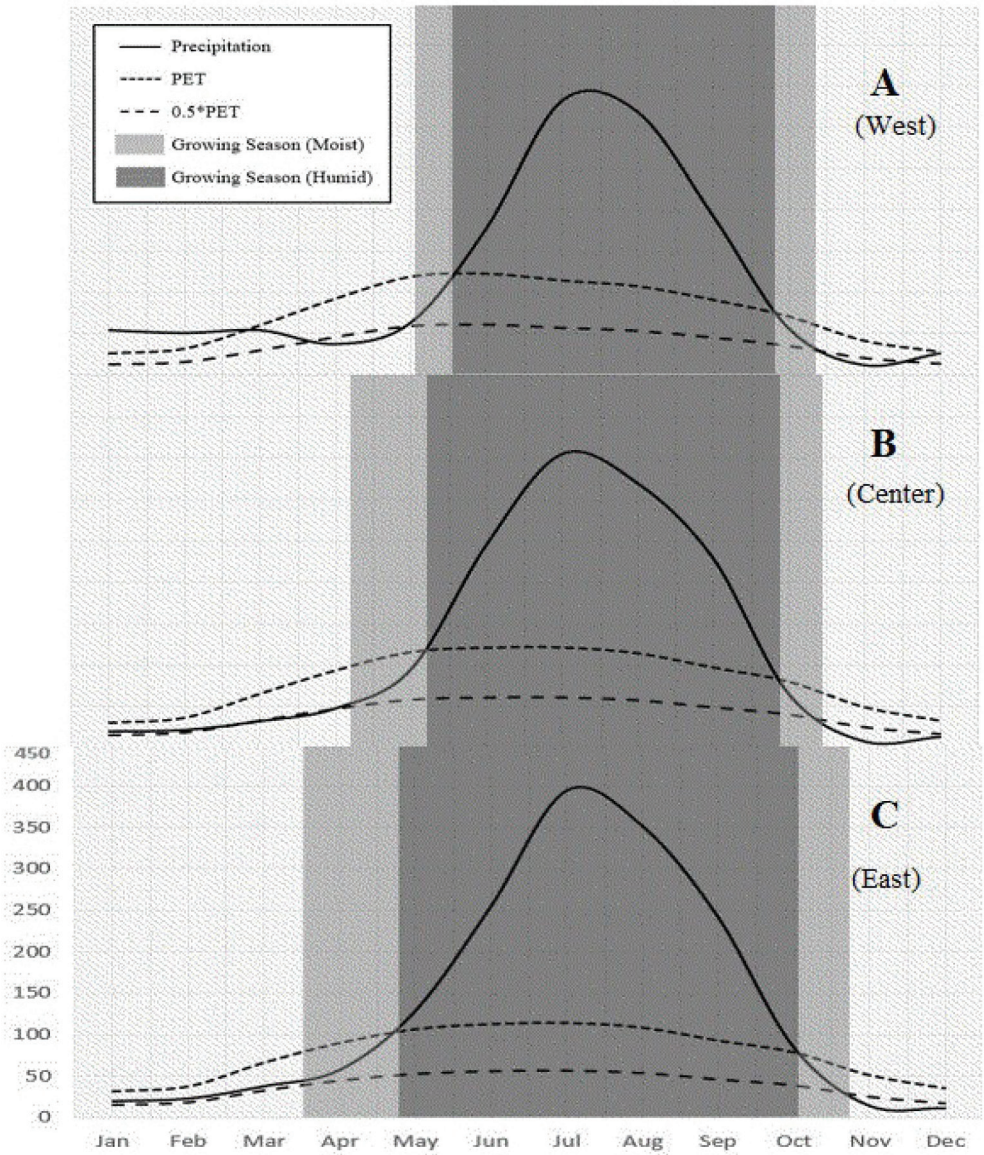


**Figure 4:** Variation in precipitation trends in different ecological regions of Nepal.

This distribution trend might have been governed by the variation in precipitation trend, length of growing seasons and temperature during summer in different ecological regions of Nepal. There is an increasing precipitation trend in Eastern Nepal (Figure 4) whereas Central and Western Nepal have decreasing precipitation trends (Pokharel *et al.,* 2019). Since Bijaysal is a deciduous tree species that requires normal precipitation (Troup, 1921), excessive rainfall can damage the seedlings resulting in water logged soil. Together, this could also be due to the variation in length of growing season in the three ecological zones. Western Nepal has shorter length of growing season as compared to Central and Eastern Nepal (Figure 3).

The present study revealed the potential distributional range of *P. marsupium* in foothills and Tarai of Western and Central Nepal. The study shows that most of the suitable habitat lies in Siwalik and Tarai region of Western Nepal compared to Central and Eastern Nepal. Restricted distribution of Bijaysal is found with high suitability in Western Nepal, moderate suitability in Central Nepal and no suitability in Eastern Nepal. Results will help in fulfilling the gaps regarding the existing population distribution in natural habitats of this species. These findings will be helpful to identify the potential habitats in the areas previously not explored, which might help in the conservation of Bijaysal in the wild and also in its reintroduction into suitable habitats.

# CHAPTER - 6
# ECOLOGY AND POPULATION STATUS: A CASE STUDY IN GWALABARI COMMUNITY FOREST, KANCHANPUR, NEPAL

**Neelam Pandey and Suresh Kumar Ghimire**

*Pterocarpus marsupium* Roxb. is a deciduous tropical tree species in the family Fabaceae. It is native to Bangladesh, India, Nepal, Sri Lanka, and Taiwan (officially the Republic of China) (Barstow, 2017). It is found mainly in lowland tropical areas, at elevations from 100 to 500 m, but exceptionally reaches up to 1200 m (Troup, 1921; Sukhadiya *et al*., 2019; Ghosh *et al*., 2021). The tree is slow growing, attaining a height up to 33 m, with spreading branches (Figure 1; Barstow, 2017). The tree can blossom and bear fruit (pod) when reaching to 5–6 years of age (Xu *et al*., 2016). The plant produces winged pods, each containing single seed (Figure 2). The tree has very limited natural regeneration due to low seed viability and poor seed germination mainly because of hard seed coat and lack of adequate exposure and sunlight in the dense tropical deciduous forest (Kalimuthu and Lakshmanan, 1995).

*P. marsupium* is a multipurpose tree, highly valued for medicine and as an excellent timber (Gairola *et al*., 2010; Sukhadiya *et al*., 2019). The older trees, when wounded, exude a blood red gum resin known as 'Kino', which mainly contains kinotannic acid and is used as an astringent (Troup, 1921; Gairola *et al*., 2010). It has suffered from overexploitation for timber (handicrafts), medicine (kino gum and bark), and fodder (DoF, 2018; Ahmad and Anis, 2019; Khanal and Bhattarai, 2020) resulting in sharp decline of natural populations globally (Anis *et al.,* 2005; Barstow, 2017). It has been listed in the Red Data book as 'Near Threatened' by IUCN based on the threats present to the species (Barstow, 2017). However, it has been argued that *P. marsupium* almost meets the criteria for 'Vulnerable' category (Barstow, 2017).

In Nepal, *P. marsupium* is restricted towards the western Tarai and Siwalik region where it occurs in small fragmented populations (DoF, 2018). Government of Nepal, under the Forest regulation 1995, banned *P. marsupium* for felling, transportation and export (MoFSC, 2002). *P. marsupium* has also been considered as 'Critically Endangered' for Nepal under CAMP (Conservation Assessment and Management Prioritization) threat category (Tandon *et al*., 2001).

The restricted range of distribution, along with its slow growth, poor regeneration, and over-exploitation pose serious threat to the exiting wild populations (Tiwari *et al*., 2004). This species needs immediate conservation effort, which should be based on a detailed knowledge about its current status. Therefore, the chapter deals with the ecology and population status of *P. marsupium* as a case study in Gwalabari Community Forest, Kanchanpur District, Nepal.

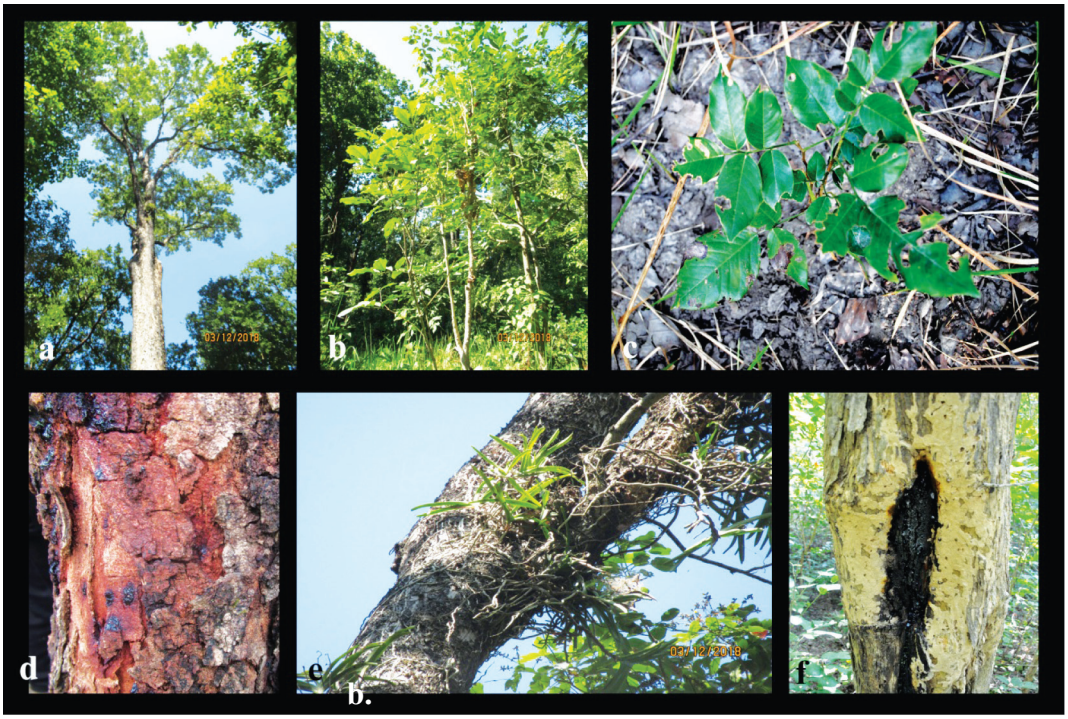


**Figure 1:** *Pterocarpus marsupium* Roxb. **a.** Adult reproductive; **b.** Adult vegetative; **c.** Young sapling; **d.** Trunk with splashed bark releasing Kino gum; **e.** Epiphytic orchid on *P. marsupium;* and **f**. Termite nest on *P. marsupium* (**Photos:** Neelam Pandey).

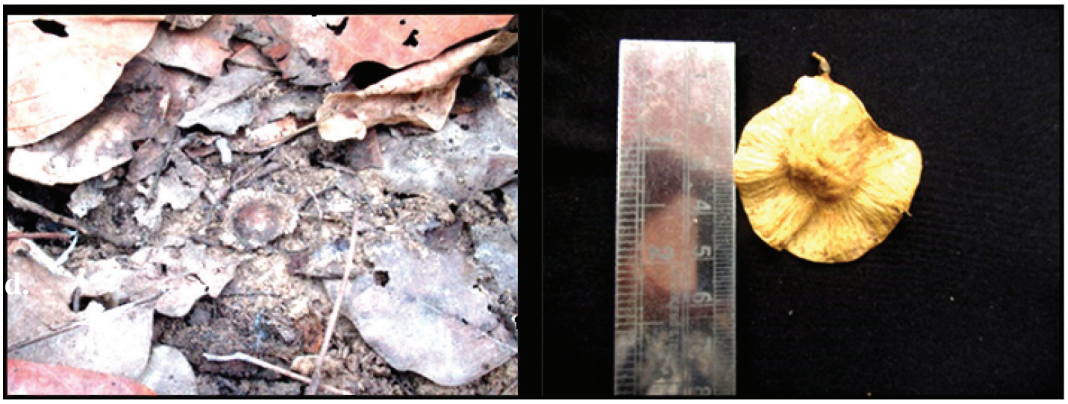

**Figure 2:** Winged pod of *Pterocarpus marsupium* (**Photos:** Neelam Pandey).

## Materials and Methods

The Gwalabari Community Forest (28°52' to 28°53'N latitudes and 80°25' to 80°26'E longitudes) is located in the Krishnapur municipality of Kanchanpur district, Sudurpaschim province, Nepal (Figure 3). The total area of the community forest is 253.44 ha. The elevation ranges from 170 to 215 m above sea level. The nearest village to the community forest comprises approximately 500 households of the

native Tharu tribe with most households being dependent on forest for fuelwood, fodder, vegetables and other non-timber forest products (NTFPs).

The entire area of the community forest was surveyed in November, 2019 to enumerate individuals of *P. marsupium* for evaluating its population status. All individuals of the target species with >10 cm DBH (diameter at breast height) were categorized as reproductive adults (Xu *et al*., 2016). Individuals with DBH ≥3–10cm were categorized as vegetative adults (Ankalaih *et al.,* 2017), and those with <3cm DBH were categorized as recruits representing saplings and seedlings (Tiwari *et al*., 2010; Kala and Dubey, 2012). Local people (*n*= 50) were interviewed to understand the use pattern of the target species and its associated impact. Habitat preference of *P. marsupium* was assessed by recording forest types and enumerating vascular plant species associated with the forest types and the target species. In addition, soil samples (at the depth of 15 cm) under the canopy of each adult tree of *P. marsupium* were collected, air dried and analyzed for pH using an electronic pH meter. Assessment of disturbance was made based on the presence of human and animal trampling, browsed plants, broken aerial parts and logged and lopped trees. Moreover, *P. marsupium* trees were observed for any kind of ecological association with other life forms.

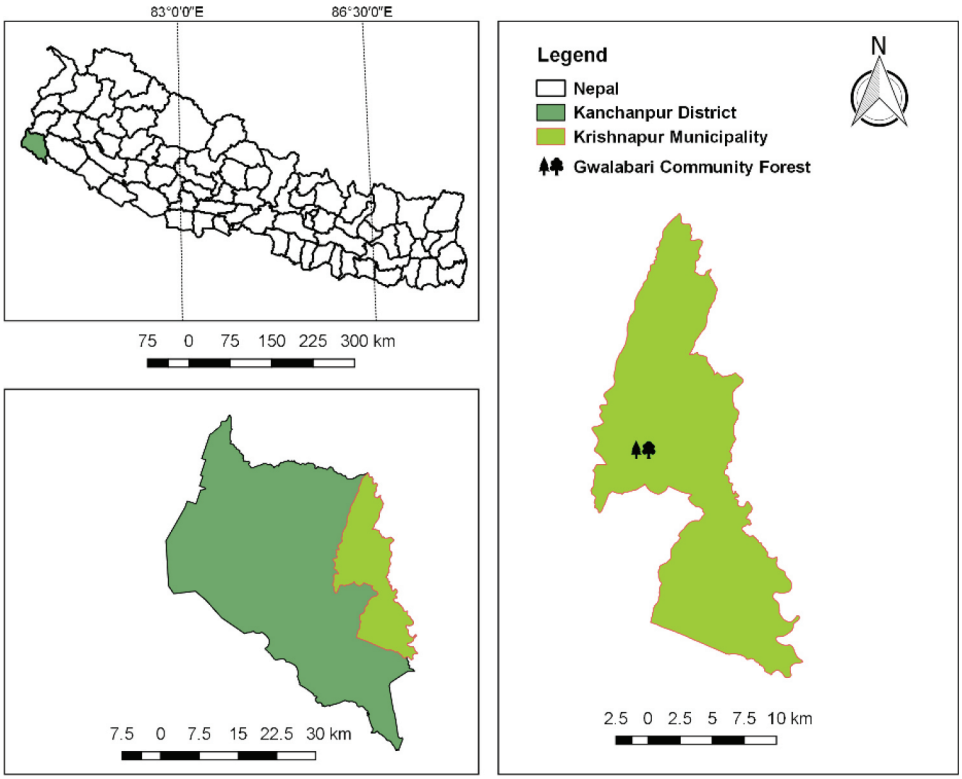


**Figure 3:** Map of the study area showing Gwalabari Community Forest, Bani, Kanchanpur.

**Population density, Regeneration status, and Level of disturbance**

A total of 177 *P. marsupium* individuals were recorded in the entire study area, representing adult reproductive (17 number of individuals), adult vegetative (49), sapling (102) and seedling (9) stages, which almost exhibited a unimodal frequency distribution (Figure 4), agreeing to the findings of previous studies (Pyakurel and Oli, 2014; Khanal and Bhattarai, 2020). While assessing the status of *P. marsupium*, Pyakurel and Oli (2014) revealed that the number of seedlings was low in most of the surveyed community forests of Kanchanpur district, Nepal. Furthermore, high proportions of poles (68%) and saplings (28%), but with few adults (3%) and no seedlings of *P. marsupium* were recorded by Khanal and Bhattarai (2020) in Hariyali Community Forest, Kapilvastu, Nepal. These results suggest that the recruitment of *P. marsupium* in western Tarai is quite low. This may be due to reduced density of reproductive individuals and low seed germination and seedling recruitment potentialities (Ankalaiah *et al*., 2017; Sukhadiya *et al*., 2019).

*P. marsupium* produces winged pods, which are 2.5–5 cm across, each containing single seed. Seeds are very light, the average dry weight of five matured seeds was calculated as $0.32 \pm 0.02$ gm ($n$ =17). Most of the seeds can be carried long distances by the wind. However, we did not observe sufficient recruitment, and this might be related mostly to the lower density of reproductive adults. Only 17 reproductive trees were recorded in this study for the entire 253.44 ha. forest area, comparable with similar findings from other parts of Nepal (e.g., Khanal and Bhattarai, 2020), can be regarded as quite low as compared to the studies from some of the tropical forests in India (e.g., Sundarapandian and Swamy, 2013; Nag and Gupta Joshi, 2020). The reduced density of matured reproductive trees may affect the seed output, ultimately affecting the future recruitment (Ankalaiah *et al*., 2017).

The adult trees were subjected to logging for timber, lopping for fodder and bark splashing and wounding practices for kino gum extraction (Figure 1). Browsed, broken or defoliated aerial parts of many seedlings were observed, indicating trampling being prevalent. Most of the adult reproductive trees ($n$ = 12) were found to be lopped for fodder or logged for heartwood. Similarly, approximately 50% of adult vegetative individuals were heavily lopped for fodder. Almost 60% of the respondents ($n$= 50) of Tharu community residing adjacent to the community forest were found to be involved in the kino gum extraction. The gum collected mainly during summer season was used as a traditional anti-diabetic medicine and also as a cooling agent. Furthermore, it was also reported to be used for joint pain and fever.

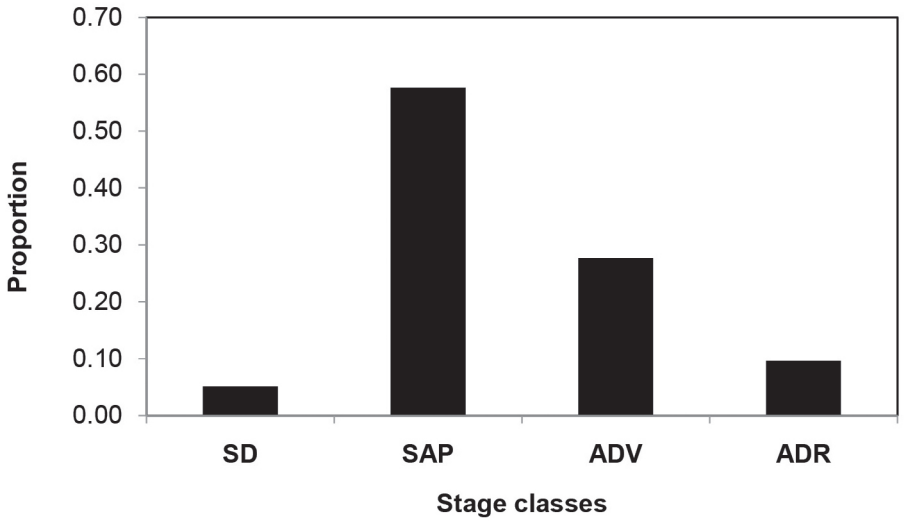


**Figure 4:** Population structure of *P. marsupium* in Gwalabari Community Forest, Kanchanpur, Nepal. Data shown are proportions of seedling (SD), sapling (SAP), adult vegetative (ADV) and adult reproductive (ADR) stages.

## Habitat preferences and other Biotic interactions

*P. marsupium* preferred weakly acidic soil (pH 6.51 ± 0.83) and the soil type was sandy alluvial and clayey. These results are consistent with the findings of Pyakurel and Oli (2014). *Shorea- Terminalia, Shorea- Mallotus*, and *Shorea- Acacia* forests are the main vegetation types found in Gwalabari Community Forest. Altogether, 106 species of vascular plants belonging to 90 genera and 37 families were found to be associated with *P. marsupium.* Fabaceae with 19 species was the dominant family associated with *P. marsupium*, followed by Lamiaceae (9 species), Malvaceae (8), Poaceae (8), Asteraceae (7) and others. *Shorea robusta* was the most frequently observed tree species, followed by *Schleichera oleosa* and *Terminalia alata.* Similarly, *Phyllodium pulchellum*, *Desmodium gangeticum, Woodfordia fruticosa*, *Mazus pumilus* and *Knoxia sumatrensis* were among the dominant shrubs and herbs associated with *P. marsupium.*

In the present study, *P. marsupium* was found being the host for epiphytic orchids like *Vanda tessellata* and *Pelatantheria insectifera* (Figure 1). Moreover, during flowering period, there was the presence of termite nest (Figure 1) on each flowering individual of *P. marsupium* indicating some kind of mutualism probably related to pollination (Ahmad *et al*., 2018). Recent study of Pal and Mondal (2018) has shown that bees (*Apis* sp.) and ants (*Camponotus* sp.) of order Hymenoptera, butterflies (species belonging to the genera *Borbo*, *Catopsilia*, *Delias*, *Euploea* and *Pachliopta*) of Lepidoptera and thrips belonging to the order Thysanoptera are the insects to visit flowers of *P. marsupium*. The association that we observed with termite is a subject of

further study. However, this observation added important insights to the postulation that *P. marsupium* is one of the important components of tropical forest with high ecological significance, which provide food to bees, ants, birds and mammals (Pyakurel and Oli, 2014; Pal and Mondal, 2018). The tree has also been regarded as a "vulture's vantage point and the climax partner of *Shorea robusta*" (DoF, 2018).

## Conclusions and Recommendations

*P. marsupium* in Gwalabari community forest comprises a very small population with lower proportions of seedlings and reproductive individuals and highest proportion of saplings. The loss of reproductive stage indicates intense current harvesting of adults. Therefore, to enhance fitness and ensure long-term viability, the existing population of *P. marsupium* should be strictly protected from harvesting, overgrazing and other human interferences. Particularly, protection and monitoring of seedlings and adult reproductive trees in the natural habitat is the immediate need for its conservation. Such monitoring should also take into consideration all the ecological factors affecting population fitness. Establishment of a system for sustainable Kino gum extraction is another important aspect to be focused. In addition, complementary approaches, such as augmentation and reintroduction programs are needed to increase the size of the existing populations and enhance its gene pool; and create new populations in the ecologically suitable site within its historical range where the species no longer occurs. Our study also provides some insights about the habitat preferences and other biotic interactions highlighting the significance of the species to the ecosystem.

# Chapter - 7
# PRETREATMENT METHODS FOR SEED GERMINATION

**Ram Krishna Bhandari and Pratikshya Chalise**

*Pterocarpus marsupium* is an epigeal germinating plant and its seeds prefer moist environment and needs loose weed free soil as well as light shade during germination (Troup, 1921; DoF, 2018). It grows best in deep, well drained, less fertile soils and can tolerate dry spells (Barstow, 2017). The suitable habitat is tropical region where plants often grow taller than the Sal (*Shorea robusta*) that enable wide and distant dispersal of seeds, which is further aided by its light, winged seed (DoF, 2018). But the propagation of *Pterocarpus* species through seeds is not much successful because of poor seed viability (Vikaspedia, 2021). However, in natural conditions, when the pods are lying uncovered on the ground surface, the radicles dry up when exposed to the intense sunlight, and are susceptible to insect damage. Similarly, the seedlings are invariably killed by frost during winter (Troup, 1921). Owing to the hardness of the pods, the seeds possess mechanical dormancy so that germination of seeds is a matter of difficulty and thus, the percentage of success is also comparatively low (Barmukh and Nikam, 2008). Besides this, poor pod set and delayed pod maturity is also another reason (Dayanand and Lohidas, 1988).

Studies have also shown that the regeneration rate of leguminous trees in natural habitats (Acharya *et al.,* 2012) as well as through tissue culture is quite low (Lakshmi *et al*., 1992; Dewan *et al*., 1992; Das and Chatterjee, 1993; Kalimuthu and Lakshmanan, 1995). In tissue culture, rooting is very scarse due to which *in vitro* propagation is seen to be less effective. Regarding natural regeneration, there are a number of factors such as prolonged juvenile period, long time duration to reach seed-bearing age, hardness of pod, low germination potential, poor seed viability due to which the seed germination percentage is lower than 30% (Venkataramaiah *et al*., 1980; Kalimuthu and Lakshaman, 1995). Poor natural regeneration is not only due to low germination and prolonged dormancy but also because of fungal growth inside the seed coat and seasonal fruit-bearing habit of the plant itself (Kumarasinghe *et al*., 2003). However, sprouting from roots can take place after the existing plants are damaged by fire (Anuradha *et al*., 2019).

After a series of struggle, some seeds hardly germinate and sprout into seedlings, but these seedlings are again limited by a number of underlying factors such as high temperature, water stress, predation, pod size, light intensity and seasonal variations. The physical nature of pods as well as seeds may also result into poor natural regeneration of the seedlings (Anuradha *et al*., 2019). Seedlings obtained via tissue culture technique also die out quickly and their survival rate is hardly 10% in open field due to intolerance of high temperature (Vikaspedia, 2021). The

cumulative effect of all these factors in turn drives this species towards an unknown bottle-neck resulting poor natural occurrences and a declining population of Bijaysal in Nepal as well as worldwide. This chapter deals with several pretreatment methods for seed germination in Bijaysal so as to propose a cost effective method for its seed germination, propagation and commercial cultivation.

## Materials and Methods

The present study was carried out in Dhakeri Botanical Garden, Nepalgunj. Pods from a healthy, matured plant were collected. Three consecutive series of experiments were carried out using different soil/ substrate types and seed pretreatment condition.

First experiment was carried out with seven different soil types and two conditions of seeds pretreatment. Seeds were soaked in lukewarm water for 24 hours as well as in normal tap water (room temperature) for 24 hours before sowing. Altogether, 14 experimental set up were carried out, each using 25 seeds (Table 1). The seeds were sown in each soil type placed in polytubes of size 4x7 on 16$^{th}$ April, 2021 and Khar (*Eulaliopsis binata*) was used for mulching the experimental set up. Each of the experimental set up was irrigated on daily basis and germination of the seeds in each set up was recorded.

**Table 1.** First experimental set up for seed germination in *P. marsupium.*

| S.No. | Soil/Substrate type | Seeds pretreatment |
|---|---|---|
| 1. | Pure soil | Treated in normal tap water for 24 hrs. |
| 2. | Soil+ Compost manure (1:1) | Treated in normal tap water for 24 hrs. |
| 3. | Pure sand | Treated in normal tap water for 24 hrs. |
| 4. | Sand+ Compost manure (1:1) | Treated in normal tap water for 24 hrs. |
| 5. | Soil+ Sand+ Compost manure (1:1:1) | Treated in normal tap water for 24 hrs. |
| 6. | Soil+ Urea+ Potash | Treated in normal tap water for 24 hrs. |
| 7. | Sand+ Urea+ Potash | Treated in normal tap water for 24 hrs. |
| 8. | Pure soil | Treated in lukewarm water for 24 hrs. |
| 9. | Soil+ Compost manure (1:1) | Treated in lukewarm water for 24 hrs. |
| 10. | Pure sand | Treated in lukewarm water for 24 hrs. |
| 11. | Sand+ Compost manure (1:1) | Treated in lukewarm water for 24 hrs. |
| 12. | Soil+ Sand+ Compost manure (1:1:1) | Treated in lukewarm water for 24 hrs. |
| 13. | Soil+ Urea+ Potash | Treated in lukewarm water for 24 hrs. |
| 14. | Sand+ Urea+ Potash | Treated in lukewarm water for 24 hrs. |

Second experiment was carried out with four different soil types and two conditions of seed pre-treatment. In first pretreatment condition seeds were soaked in normal tap water for 18 hours and in second pretreatment condition seedcase were incised but not treated in water. In first pretreatment condition, the seeds that floated in the surface of water and those which shrunk underneath were treated separately. Altogether, 12 experimental set up were carried out, each using 50 seeds (Table 2). The seeds were sown in each soil type prepared in nursery beds on 2nd May, 2021 and germination of the seeds in each set up was recorded.

**Table 2.** Second experimental set up for seed germination in *P. marsupium.*

| S.No. | Soil/Substrate type | Seeds pretreatment |
|---|---|---|
| 1. | Pure soil | Treated in normal tap water for 18 hrs, sunken seeds only considered |
| 2. | Soil+ Compost manure (1:1) | Treated in normal tap water for 18 hrs, sunken seeds only considered |
| 3. | Soil+ Sand+ Compost manure (1:1:1) | Treated in normal tap water for 18 hrs, sunken seeds only considered |
| 4. | Soil+ Urea+ Potash | Treated in normal tap water for 18 hrs, sunken seeds only considered |
| 5. | Pure soil | Treated in normal tap water for 18 hrs, floated seeds only considered |
| 6. | Soil+ Compost manure (1:1) | Treated in normal tap water for 18 hrs, floated seeds only considered |
| 7. | Soil+ Sand+ Compost manure (1:1:1) | Treated in normal tap water for 18 hrs, floated seeds only considered |
| 8. | Soil+ Urea+ Potash | Treated in normal tap water for 18 hrs, floated seeds only considered |
| 9. | Pure soil | Seedcase incised but not treated in water |
| 10. | Soil+ Compost manure (1:1) | Seedcase incised but not treated in water |
| 11. | Soil+ Sand+ Compost manure (1:1:1) | Seedcase incised but not treated in water |
| 12. | Soil+ Urea+ Potash | Seedcase incised but not treated in water |

Third experiment was carried out with five different soil types and two conditions of seed pre-treatment. Here, instead of seeds the whole pods were soaked in normal tap water for 24 hours and the seeds were extracted from the soaked pods and floatation test was carried out. In first pretreatment condition, the seeds that floated in the surface of water were considered and in second pre-treatment condition, the seeds that shrunk underneath were considered. Altogether, eight experimental set up were carried out, each using 50 seeds (Table 3). The seeds were sown in each soil type placed in polytubes of size 4x7 on 21st May, 2021 and germination of the seeds in each set up was recorded.

**Table 3.** Third experimental set up for seed germination in *P. marsupium.*

| S.No. | Soil/Substrate type | Seeds pretreatment |
|---|---|---|
| 1. | Pure soil | Pods treated in normal tap water water for 24 hrs, sunken seeds only considered |
| 2. | Soil+ Compost manure (1:1) | Pods treated in normal tap water water for 24 hrs, sunken seeds only considered |
| 3. | Soil+ Sand+ Compost manure (1:1:1) | Pods treated in normal tap water water for 24 hrs, sunken seeds only considered. |
| 4. | Sand+ Compost manure (1:1:1) | Pods treated in normal tap water water for 24 hrs, sunken seeds only considered |
| 5. | Pure soil | Pods treated in normal tap water for 24 hrs, floated seeds only considered |
| 6. | Soil+ Compost manure (1:1) | Pods treated in normal tap water for 24 hrs, floated seeds only considered |
| 7. | Soil+ Sand+ Compost manure (1:1:1) | Pods treated in normal tap water for 24 hrs, floated seeds only considered |
| 8. | Sand+ Compost manure (1:1:1) | Pods treated in normal tap water for 24 hrs, floated seeds only considered |

**All the pods were soaked in normal tap water for 24 hours and the seeds were extracted from the soaked pods.*

## Seed germination experiment

Seed germination took place between 5 to 25 days of sowing. Although extraction of seeds was a very tedious task, germination was faster while using the seeds rather than using the pods. Treatment of seeds with cold water (at room temperature) was an efficient method for the seed germination. Here, only the sunken seeds germinated and germination took place between 5 to 25 days. However, in case of the seeds that floated in water during pretreatment, germination was absent. Similarly, in the seeds pretreated with luke warm water, germination was absent. Mulching with Khar during the first set up, increased the infestation of termites which destroyed the seeds as well as germinating seedlings. Similarly, excessive irrigation into the polytubes also destroyed the seedlings. Germination percentage was maximum (30%) when combination of soil, sand and compost (1:1:1) was used as the substrate/soil type and the seeds were treated with cold water for 24 hours and only sunken seeds were considered for sowing. The result of the germination study on the three consecutive experiments is given in the Table 4, 5 and 6.

**Table 4.** Result of seed germination from the first experimental set up.

| S. No. | Soil/substrate type | Seeds pretreatment | No. of seeds | Germinated seeds | Remarks |
|---|---|---|---|---|---|
| 1 | Pure soil | Normal tap water | 25 | 1 | Germinated on 23rd April, 2021. (Leaf tip turned black and died on 2nd day) |
| 2 | Soil+ Compost manure | Normal tap water | 25 | 0 | - |
| 3 | Pure sand | Normal tap water | 25 | 0 | - |
| 4 | Sand+ Compost manure | Normal tap water | 25 | 0 | - |
| 5 | Soil+ Sand+ Compost manure | Normal tap water | 25 | 1 | Germinated on 23rd April, 2021. Later destroyed by heavy rain and hail |
| 6 | Soil+ Urea+ Potash | Normal tap water | 25 | 0 | - |
| 7 | Sand+ Urea+ Potash | Normal tap water | 25 | 0 | - |
| 8 | Pure soil | Luke warm water | 25 | 0 | Seeds could not germinate when treated with Luke warm water. |
| 9 | Soil+ Compost manure | Luke warm water | 25 | 0 | |
| 10 | Pure sand | Luke warm water | 25 | 0 | |
| 11 | Sand+ Compost manure | Luke warm water | 25 | 0 | |
| 12 | Soil+ Sand+ Compost manure | Luke warm water | 25 | 0 | |
| 13 | Soil+ Urea+ Potash | Luke warm water | 25 | 0 | |
| 14 | Sand+ Urea+ Potash | Luke warm water | 25 | 0 | |

**Table 5.** Result of seed germination from the second experimental set up.

| S. No. | Soil/ Substrate type | Seeds pretreatment | No. of seeds | Germinated seeds | Germination (%) | Remarks |
|---|---|---|---|---|---|---|
| 1 | Pure soil | Normal tap water / sunken seeds | 50 | 4 | 8 | 5 seeds germinated between 5 to 12 days after sowing. But 1 seedling died by 20$^{th}$ day. |
| 2 | Soil+ Compost manure | Normal tap water / sunken seeds | 50 | 7 | **14** | Seeds germinated between 5 to 17 days after sowing. All the seedlings survived. |

| S. No. | Soil/ Substrate type | Seeds pretreatment | No. of seeds | Germinated seeds | Germination (%) | Remarks |
|---|---|---|---|---|---|---|
| 3 | Soil+ Sand+ Compost manure | Normal tap water / sunken seeds | 50 | 2 | 4 | Seeds germinated between 12 to 17 days after sowing. |
| 4 | Soil+ Urea+ Potash | Normal tap water / sunken seeds | 50 | 1 | 2 | 3 seeds germinated within 12 days. But two seedlings died on 20$^{th}$ day. |
| 5 | Pure soil | Normal tap water / floated seeds | 50 | 0 | 0 | Floated seeds did not germinate. |
| 6 | Soil+ Compost manure | Normal tap water / floated seeds | 50 | 0 | 0 | |
| 7 | Soil+ Sand+ Compost manure | Normal tap water / floated seeds | 50 | 0 | 0 | |
| 8 | Soil+ Urea+ Potash | Normal tap water / floated seeds | 50 | 0 | 0 | |
| 9 | Pure soil | Seedcase incised/ no water treatment | 50 | 1 | 2 | Seeds germinated 12 days after sowing. |
| 10 | Soil+ Compost manure | Seedcase incised/ no cold water treatment | 50 | 0 | 0 | - |
| 11 | Soil+ Sand+ Compost manure | Seedcase incised/ no water treatment | 50 | 1 | 2 | Seeds germinated 17 days after sowing. |
| 12 | Soil+ Urea+ Potash | Seedcase incised/ no water treatment | 50 | 1 | 2 | 3 seeds germinated 12 days after sowing. But 2 seedlings died within the 1$^{st}$ week. |

** All the seedlings were carefully transplanted from the nursery beds to polytubes on 4$^{th}$ June, 2021, i.e, after 28 days.*

**Table 6.** Result of seed germination from the third experimental set up.

| S.No. | Soil/ Substrate type | Seeds/ Fruits pretreatment | No. of seeds | Germinated seeds | Germination (%) | Remarks |
|---|---|---|---|---|---|---|
| 1 | Pure soil | Normal tap water/ sunken seeds | 50 | 5 | 10 | Seeds germinated between 5 to 15 days after sowing. |
| 2 | Soil+ Compost manure | Normal tap water/ sunken seeds | 50 | 4 | 8 | Seeds germinated between 10 to 20 days after sowing. |
| 3 | Soil+ Sand+ Compost manure | Normal tap water/ sunken seeds | 50 | 15 | 30 | Seeds germinated between 5 to 25 days after sowing. |
| 4 | Sand+ Compost manure | Normal tap water/ sunken seeds | 50 | 6 | 12 | Seeds germinated between 5 to 15 days after sowing. |
| 5 | Pure soil | Normal tap water/ floated seeds | 50 | 0 | 0 | Floated seeds did not germinate. |
| 6 | Soil+ Compost manure | Normal tap water/ floated seeds | 50 | 0 | 0 | |
| 7 | Soil+ Sand+ Compost manure | Normal tap water/ floated seeds | 50 | 0 | 0 | |
| 8 | Sand+ Compost manure | Normal tap water/ floated seeds | 50 | 0 | 0 | |

** All the seeds that germinated survived during the third experiment.*

Gamble (1972) reported that seeds of Bijaysal do not always germinate very well. Germination is better when the seeds are taken out from the pod before sowing, but extracting the true seeds from the pod was a tedious and labor intensive task. Barmukh and Nikam (2008) also reported that seeds are prone to injury or, physical damage while separating from the pods. Here, seed extraction was easier when the pods were soaked in water for 24 hours before sowing. This also enhanced the seed germination percentage.

Seeds of *Pterocarpus marsupium* germinated between 5 to 25 days. Similar time of germination was reported by Van Dalean (1991) where seed germination took place between one to six weeks. Seedlings initially have simple, unifoliate leaves which later developed into compound, imparipinnate leaves (Figure 1).

**Factors affecting Seed germination**

Seed germination depend on several factors such as time of fruit harvest (Chauhan *et al.,* 2018), extraction of seeds, seed pretreatment, light, moisture (water supply/ irrigation), insect infestation. During first experiment, germination was affected by the time of pod harvesting as well as seed sowing. Here, the pods were harvested during first week and sown during second week of April, exhibiting very little germination. This might be because seed germination increases with seed maturity (Ahmad *et al.,* 2015). Together, mulching was done which reduced the availability of light and increased the possible infestation of termites. Excess irrigation also suppressed the germination.


**Figure 1:** Study of seed germination in *Pterocarpus marsupium*; **a.** Seeds after extraction from pods; **b.** Seeds; **c.** Floatation test of seeds; **d.** Germinating seed; **e.** Seedling with two cotyledonary leaves; **f.** Seedling with two cotyledonary leaves and one true leaf; **g.** Seedling with two cotyledonary leaves and a pair of true leaves; **h.** Seedling with two cotyledonary leaves and three true leaves; **i.** Seedling with two cotyledonary leaves and four true leaves; **j.** Seedling with degenerating cotyledonary leaves and five true leaves; and **k.** Seedlings of Bijaysal in 4X7 polytubes. (**Photos:** Ram Krishna Bhandari).

Seed germination was higher when the seeds were pretreated with normal tap water for 24 hours. Vikaspedia (2021) also reported that germination is enhanced when the seeds are treated with water before sowing. The seeds that shrunk underneath had higher germination percentage compared to the seeds that float in water, in which germination was completely absent. This might be because, seeds with high specific gravity, larger size, good quality and viability shrunk underneath whereas the seeds with low specific gravity, small size or, immature, poor quality, poor viability or, nonviable seeds float on water. However, the seeds that were treated in lukewarm water for 24 hours, did not germinate. The seed coat is not very hard but the hardness is due to their pods; which after separating make the seeds susceptible to damage by heat as well.

The plant does not require excess water if the seeds are treated with water before sowing. Mulching is also not necessary as mulching increased insect infestation. However, protecting the germinating seedlings from excess heat as well as environmental extremes such as hail, thunderstorm, etc. are required for better growth.

Germination was comparatively higher when whole pods were pretreated with normal tap water for 24 hours and only the sunken seeds were sown in substrate containing equal proportion of soil, sand and compost manure. Troup (1921) also reported that propagation of the plant can also be done by soaking the pods for few days before sowing them into the ground. In this case, the margins of the pods should be cut so that water enters inside the pods and are ultimately used up by the seeds. This could be a fast, cost effective pretreatment method for the germination of the seeds; which could provide new insights for the propagation as well as commercial cultivation of Bijaysal in context of Nepal. However, there are several other techniques for germination and propagation of tree species such as treatment with acids in different concentration, physical scarification, heat treatment, etc. which were not considered in this study, so further studies can be carried out for testing their validity.

# CHAPTER - 8
# ECONOMIC BOTANY AND ETHNOBOTANY

**Pratikshya Chalise and Sajita Dhakal**

*Pterocarpus marsupium* is a versatile plant with a broad spectrum of uses (Troup, 1921) that comprise of its medicinal, fodder, and timber values (Acharya *et al.*, 2002). It has been continuously mentioned in various traditional systems of medicine like Ayurvedic, Unani and Homeopathic systems of medicine (Badkhane *et al.*, 2010). Almost every parts of the plant are used as primitive home remedies against various human diseases (Manne *et al.*, 2020; Prasad *et al.*, 2007). Pant and Yadav (2013) also reported Bijaysal to have high medicinal value and listed it among the 20 most exploited plant species in Kanchanpur district, Western Nepal. Wood is very hard and durable so it is considered as a multipurpose tree throughout India (Troup, 1921; Troup, 1986). Wooden tumblers made from the wood of Bijaysal tree are still used to control diabetes (Grover *et al.,* 2002; Reddy *et al*., 2008; Badkhane *et al.,* 2010; Khare, 2010; Joshi *et al.*, 2012; Khanal and Bhattarai, 2020; Vikaspedia, 2021) and is referred to as 'The miracle cure for diabetes' (Katiyar *et al.*, 2016). The water left in these tumblers overnight (Joshi *et al.*, 2012), when consumed daily twice for 30 days has shown beneficial effects in individuals suffering from diabetes (Katiyar *et al.*, 2016).

Stem bark is boiled and consumed as a tonic after child birth (Prasad *et al.,* 2007). Heartwood and kino gum are useful in the skin as well as blood-related diseases as they have astringent, anti-diarrhoeal and anti-haemorrhagic properties (Badkhane *et al*., 2010; Vikaspedia, 2021). Similarly, the leaves are used externally to treat boils, sores and other skin diseases and the flowers are used as febrifuge (Seema *et al.,* 2010; Vikaspedia, 2021). The seed paste and decoction of heartwood is used to cure Diabetic anaemia (Badkhane *et al*., 2010).

The bark of stem is often slashed for the Kino gum due to its medicinal properties. Khanal and Bhattarai (2020) reported that lower parts of the tree trunk are usually exposed and gums are collected. The synthesis of information in this chapter aims to stimulate further research on economic and ethnobotanical uses of *P*. *marsupium*.

## Materials and Methods

Present investigation was carried out based on the survey carried out in Malakheti and Khamaura villages, Kailali district and Bhimdattanagar of Kanchanpur district, Far West, Nepal during November, 2020 (Figure 1).

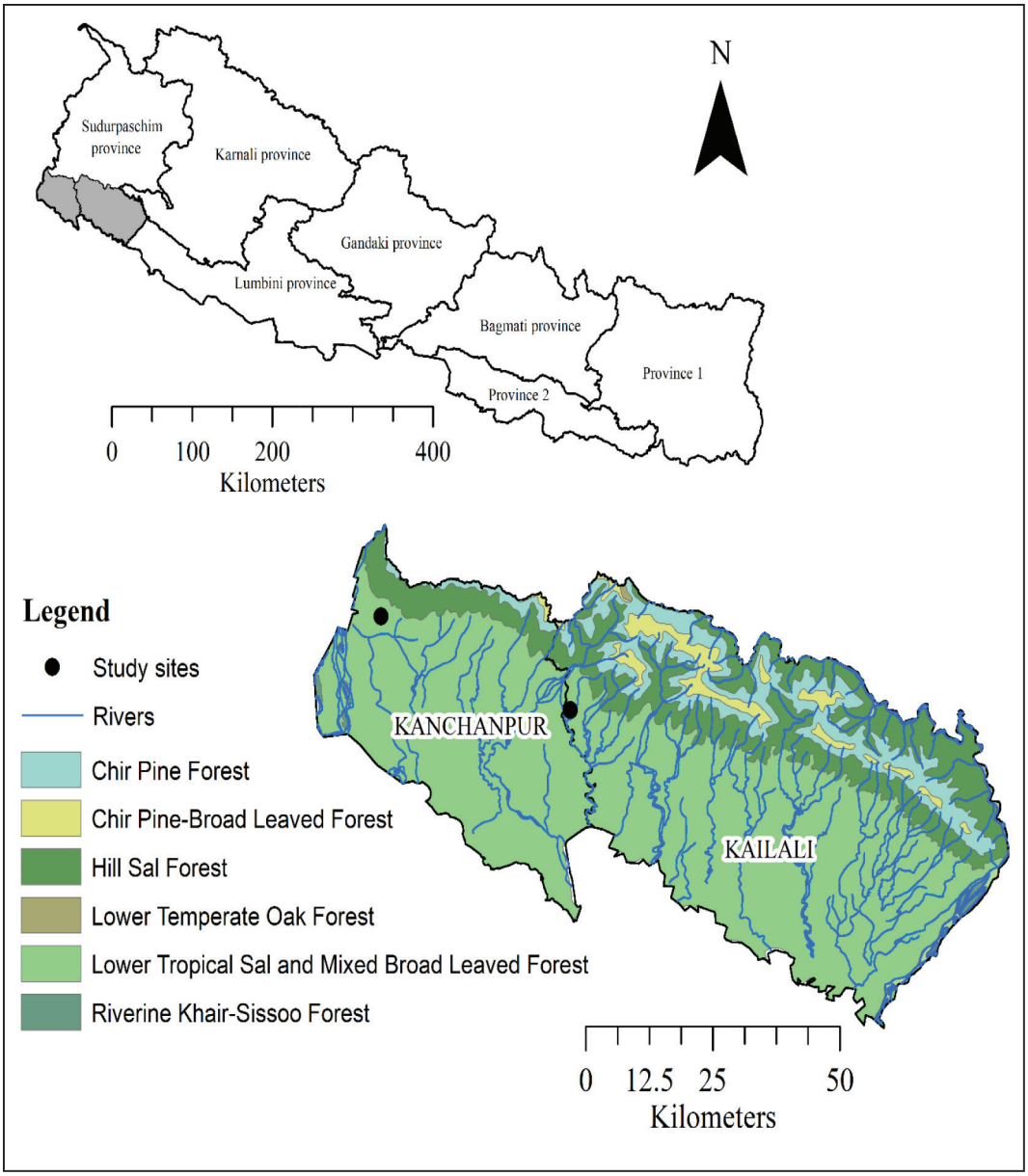


**Figure 1:** Map showing the study sites in Kailali and Kanchanpur districts of Sudurpaschim province.

Information on economic as well as ethno-botanical uses of Bijaysal was collected from the study area using questionnaire method. In both Kailali as well as Kanchanpur districts, 45 respondents with age ranged above 35 years were interviewed. In both study sites, 20 respondents were male and 25 were female. The informants were also asked to specify the parts used and the mode of using it. The major inhabitants of the study area are Brahmin, Chhetri and Tharus.

## Use Profile of Pterocarpus marsupium

Majority of the respondents interviewed mentioned Bijaysal to be an important plant species since ages. The economic as well as ethnobotanical uses of Bijaysal reported during this study are presented in table 1.

**Table 1**. Economic and ethnobotanical uses of *Pterocarpus marsupium* in Kailali and Kanchanpur districts, Far West, Nepal.

| S. No. | Parts used | Use category | Uses (against/as) | Mode of use |
|---|---|---|---|---|
| 1. | Heartwood | Medicinal | Diabetes | Water is filled in wooden tumblers and left overnight and the next morning this water is taken in empty stomach. Alternatively, wooden powder/ pieces is soaked in water overnight and the next morning the water is filtered and consumed in empty stomach. Wood is used in making ploughs, toys, furniture's, etc. |
| | | | Body ache | |
| | | | Joint pain | |
| | | | Cough and cold | |
| | | Others | Furniture | |
| 2. | Leaves | Medicinal | Skin diseases | Leaf paste is applied on the skin to cure skin problem |
| | | | Insect bites | Leaf paste is applied on the skin in case of insect bites. |
| | | Fodder | Fodder for cattles | Branches are lopped and leaves are given as fodder to domestic cattles. |
| 3. | Bark | Medicinal | Skin infection | Paste of bark is applied on the skin to cure skin infection, scabies, etc. |
| | | | Diabetes | Bark powder is soaked in water and consumed. |
| | | | Toothache | Paste of bark is also applied in case of toothache. |
| | | | Malaria and fever | Paste of bark is consumed directly. |
| | | Others | Dyes | Fresh bark is used to extract the dyes. |

| S. No. | Parts used | Use category | Uses (against/as) | Mode of use |
|---|---|---|---|---|
| 4. | Kino gum | Medicinal | Diabetes | Bark of tree are slashed and gum is collected. Kino gum is stored in small bottle and taken whenever necessary. |
| | | | Body ache | Kino gum is consumed directly. quantity |
| | | | Diarrhoea and Dysentery | Kino gum is consumed directly. |
| | | | Toothache | Paste of bark is also applied in case of toothache. |
| 5. | Flowers | Medicinal | Fever | Flowers are consumed directly to control fever. |
| 6. | Twigs and branches | Firewood | Firewood | Branches and twigs are dried properly and used as firewood. |
| | | Medicinal | Toothache | Twigs of Bijaysal are used to brush teeth due to their medicinal properties |
| 7. | Wood/ wood powder | - | - | Used as an alternative to tea leaves. |

### *Economic Aspects*

Locally the heartwood is used for making wooden tumbler that is in high demand due to its medicinal value. Several other utensils, musical instruments, cups, glasses, gift and jewelry boxes, etc. are prepared and sold in the local market. The wood powder is used as an alternative to tea and thus packets of wooden powder is also sold in the market. The bark is also used for toothache as well as for dyeing purpose (Sukhadaya *et al.*, 2019). Similarly, Troup (1921) reported that the wood is used for making buildings, agricultural equipments, carts, wheel-works, boats, etc. Khare (2007) and Sukhadaya *et al.* (2019) reported that the wood of Bijaysal is well known due to its excellent timber value and ranks next to teak and rosewood throughout the peninsular India.

### *Ethnobotanical aspects*

Locally, the residents of Western Tarai especially of Kailali and Kanchanpur districts have been using heartwood as well as Kino gum for body ache, joint pain and gastro-intestinal disorders. It is listed among the most exploited plant species in Far Western Tarai (Pant and Yadav, 2013). Traditional medical practitioners like Baidhya/ Baidawa, local healers; Guruwa, etc. are engaged in folklore medicines similar to the studies from other parts of the country (Khanal and Bhattarai, 2020). Several parts of the plant such as heartwood, bark, leaves, flowers, gum resin, etc. are used since many generations because of their inherent medicinal properties. The indigenous


**Figure 2:** *Pterocarpus marsupium*: field observation. **a.** Adult trees in the cultivable land; **b.** Local people collecting fodder for cattle; **c.** Collecting information from the local stakeholders in Kanchanpur district; **d.** Local in Kanchanpur district making wooden tumblers from heartwood Bijaysal; **e.** Making wooden tumblers; and **f.** Wooden tumblers and other wooden utensils made from Bijaysal (**Photos:** a., c., and f. Yagya Raj Paneru; b., d., and e. Pratikshya Chalise).

Tharus and Brahmins collect the Kino gum and store them in small plastic bottles and consume it whenever and wherever necessary. Several traditional ploughs and bullock carts are made from the wood of Bijaysal. But, some local healers were reluctant to share their knowledge about the medicinal plants and their properties. This is because they fear sharing their knowledge with other will make their gurus angry, and they will lose their knowledge as well as the ability to heal forever.

Similipalkol tribes in Odisha, India make a paste of the barks of *Pterocarpus marsupium*, *Mangifera indica*, *Shorea robusta* and *Spondias pinnata* to treat dysentery and other diarrheal illnesses (Sharma and Gautam, 2017). Similarly, the Kannada people of India believe that a poultice prepared from the bark and leaves of Bijaysal possesses astringent properties so it is useful in treating skin infections (Sharma and Gautam, 2017).

However, due to changing perception of the local people from generation to generation; due to the ever increasing influence of global commercialization, socio-economic transformation and modern medicinal practices; indigenous knowledge on uses of plant resources is constantly diminishing (Gadgil *et al.*, 1993; Kunwar and Adhikari, 2005).

## Conclusion and Recommendations

*P. marsupium* is an important tree species with high medicinal value; renowned as an important remedy against diabetes. However, despite traditional claims, insufficient scientific validation for the treatment of diabetes, dysentery, cough and cold, joint pain, skin diseases, fever and toothache; require further examinations. It is under threat of extinction due to increasing human pressure as well as habitat degradation due to several anthropogenic activities such as grazing, lopping, expansion of agriculture land, forest fire, etc. and improper collection of Kino gum locally. The present study endeavors to document the traditional ethnobotanical information and the overall usage of this multipurpose tree. Further, the information generated from this study will give a premise to phytochemical and pharmaceutical studies.

# CHAPTER - 9
# PHYTOCHEMISTRY, ANTIOXIDANT, ANTIDIABETIC ACTIVITIES AND TOXICITY

**Parasmani Yadav and Devi Prasad Bhandari**

*Pterocarpus marsupium* is an important therapeutic and medicinal plant (Rahman *et al.,* 2018). It is one of the most valuable multipurpose tropical tree and has great importance of producing timbers. The bark and resin decoction is an astringent for severe diarrhoea, dysentery, for the treatment of tumours of the glands, urethral discharges, on ringworm of the scalp, chronic ulcers and abortifacient (Basu *et al.,* 1975). The heart wood is astringent, bitter acrid, anti-inflammatory, anthelmintic, anodyne (Kritikar, 1987). It is also good for elephantiasis, leucoderma, diarrhoea, rectalgia, cough and greyness of hair (Mankani *et al*., 2005). Besides these, the bark of *P. marsupium* is found to be the most effective in preventing cataract development and reducing hyperglycemia (Vats *et al*., 2004). It is safe and effective in wounds, fever, stomach ache, diabetes, jaundice and ulcers (Jung *et al.,* 2006).

## Materials and Methods

The leaf and bark samples of Bijaysal were collected from the premises of Dhakeri Botanical Garden, Banke and was identified at National Herbarium and Plant Laboratories, Godawari, Lalitpur. Herbarium specimens were prepared and deposited at National Herbarium and Plant Laboratories (KATH). Samples were cleaned and shade dried before the extraction process.

### *Preparation of Plant extracts*

Crude extracts of bark and leaves were prepared by Soxhlet extraction method. 10 gm of powdered plant material was uniformly packed into a thimble and extracted with 100 ml of Methanol. The process of extraction continued for 18-24 hours. Chlorophyll was removed wherever necessary by treating the methanolic extract with Hexane in separating funnel. The extracts were concentrated by keeping beakers in water bath at 55ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.

### *Qualitative Phytochemical Analysis*

Qualitative phytochemical analysis was carried by Standard analytical procedures as described by Harbone (1973), Trease and Evans (1989), Sofowora (1993) and Aluko *et al.* (2012).

1. Test for reducing sugars (Fehling's test)

   Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

2. Test for glycoside

   4 ml of extract solution was dried till 2 ml and 1-2 ml of Ammonium hydroxide was added. The resulting mixture was shaked. Appearance of Cherish red colour indicates the presence of glycosides.

3. Salkowski's test

   Crude extract was mixed with 2 ml of Chloroform. Then 2 ml of concentrated $H_2SO_4$ was added carefully and shaked gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

4. Keller-Kilani test

   Crude extract was mixed with 2 ml of Glacial Acetic acid containing 1-2 drops of 2% $FeCl_3$ solution. The mixture was then poured into another test tube containing 2ml of concentrated $H_2SO_4$. A brown ring at the interface indicated the presence of cardiac glycosides.

5. Test for polyphenols and tannins

   Crude extract was mixed with 2 ml of 2% $FeCl_3$ solution. A blue-green or blue-black coloration indicated the presence of polyphenols and tannins.

6. Test for flavonoids

   Crude extract was mixed with few fragments of Magnesium ribbon and concentrated HCl was added dropwise. Pink or magenta red colour appeared after few minutes which indicated the presence of flavonoids.

7. Test for saponins

   Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaked vigorously for 30 seconds. The formation of stable foam (1 cm height) even after 30 minutes was taken as an indication for the presence of saponins.

8. Test for steroids

   Crude extract was mixed with 2 ml of Chloroform and concentrated $H_2SO_4$ was added sidewise. A red colour produced in the lower Chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of Chloroform. Then, 2 ml of each, concentrated $H_2SO_4$ and Acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

9. Test for terpenoids

   Crude extract was dissolved in 2 ml of Chloroform and evaporated to dryness. Then, 2 ml of concentrated $H_2SO_4$ was added; a reddish brown coloration at the interface indicated the presence of terpenoids.

10. Test for alkaloids

    Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

11. Test for coumarins

    Extract solution is concentrated to yield a residue. The residue is dissolved in hot water. After cooling, the solution is divided in two test tubes. Then, to one test tube 10% (w/v) Ammonium hydroxide is added whereas the other test tube is used as control. Fluorescence colour indicates the presence of coumarins.

***Observation***

The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

**Determination of Total Phenolic Content**

Phenolic content was measured by using the Folin–Ciocalteu reagent in each extract (Singleton *et al.,* 1999). The results were derived from a calibration curve of different concentration of Gallic acid and expressed in Gallic acid equivalents (GAE) per gram dry extract weight.

***Preparation of Reagent***

1. Preparation of Sodium carbonate solution

   1 Molar Sodium carbonate solution was prepared by dissolving 2.65g of Sodium carbonate in 25 ml distilled water, 1 ml of FCR reagent was mixed with 10 ml of water to prepare 1:10 FC reagent.

2. Preparation of Standard Gallic acid and sample solution

   5 mg Gallic acid was dissolved in 5 ml of Ethanol to prepare the stock solution of 1000 µg/ml concentration. The stock solution was further diluted into final concentration of 10, 20, 30, 40, 50, 60, 70, 80 µg/ml. Different concentration of the Gallic acid was used as positive control in total phenolic content test.

Similarly, plant extract of 5000 µg/ml concentration were prepared from stock solution of crude extract in 50% DMSO solution.

***Procedure***

Total phenolic content of both plant extract was determined by using Folin-Ciocalteu reagent. Gallic acid of different concentration 10, 20, 30, 40, 50, 60, 70, 80 µg/ml was loaded triplicate used for the standard control. Plant sample of 5000 µg/ml was loaded 20 µl triplicate. 100 µl of the FC reagent was added in each well containing Gallic acid and plant sample. Initial reading of plate was taken at 765 nm using a microplate reader. After initial reading 80 µl of $Na_2CO_3$ was added separately to each well, and incubated for 15 minutes. After incubating the plate, the final absorbance was taken 765 nm in (Epoch2, BioTek, Instruments, Inc, USA) microplate reader. Standard curve of the Gallic acid was plotted as standard curve.

**Determination of Total Flavonoid Content (TFC)**

Total flavonoid contents in selected plant extracts were determined by using Aluminium chloride in a colorimetric method (Atanassova *et al.*, 2011). The results were derived from the calibration curve of different concentration of quercetin and expressed in quercetin equivalents (QE) per gram dry extract weight.

***Preparation of Reagent***

1. Preparation of Aluminum trichloride

   10% Aluminum trichloride was prepared by dissolving 1 g of $AlCl_3$ into 10 ml distilled water and 1 M potassium acetate in 10 ml distilled water was prepared.

2. Preparation of standard Quercetin and sample solution

   1.54 mg quercetin was dissolved in 10 ml water to prepare 154 µg/ml concentration of stock solution. By diluting the stock solution of the Quercetin different concentration of the quercetin 10, 20, 40, 60, 80, 100 µg/ml was prepared. Similarly, plant sample was prepared by diluting stock solution in 50% DMSO.

***Procedure***

130 µl of different concentration (10, 20, 40, 60, 80, 100 µl) of the Quercetin was loaded triplicate in 96 well plate. Similarly, 20 µl of the plant sample (5000 µg/ml) was loaded triplicate; and 110 µl of distilled water was added in each well containing plant sample. 60 µl of Ethanol was added to each well containing plant extract and Quercetin. Initial reading was taken at wavelength 415 nm in microplate reader (Epoch 2, BioTek, Instruments, Inc. USA). After initial reading of the plate, 5-5 µl

of $AlCl_3$ was added in each plate. After addition of the reagent, plate was incubated in dark for 30 minute and final reading of plate was taken at same the wave length.

**Determination of Antioxidant activity**

The antioxidant activity was determined as DPPH Radical Scavenging Activity (Hu e*t al.*, 2011). All data were compared with the $IC_{50}$ value of standard Quercetin.

***Preparation of Reagent***

1. Preparation of DPPH solution (0.1mM)

   0.1 mM DPPH solution was prepared by dissolving 1.95 mg DPPH in 50 ml Methanol in Dark volumetric flask.

2. Preparation of quercetin solution and plant sample

   1 mg of Quercetin was dissolved in 10 mL Methanol to prepare stock solution of 100 µg/ml. Quercetin solutions of 20, 10, 5, 2.5, 1.25, 0.625 µg/ml concentration were prepared by diluting the stock solution. Similarly, plant extracts of different concentration (500, 250, 125, 62.5, 31.25 µg/ml) was prepared in 50% DMSO, from stock solution of 5000 µg/ml.

***Procedure***

Radical scavenging activity of the plant sample was evaluated by modified colorimetric method, from 96 well plate. For standard curve, Quercetin 100 µl of different concentrations (20, 10, 5, 2.5, 1.25, 0.625 µg/ml) was loaded triplicate as positive control, and 50% DMSO was loaded triplicate as negative control. Plant samples of different concentration were loaded, 100 µl to each well and initial absorbance was measured. After initial reading, 100 µl of DPPH solution was added in each well and was incubated for 30 minutes in dark. After incubation, final reading was taken and the percentage inhibition of the sample was calculated and compared with standard curve of Quercetin.

$$\% \text{ Inhibition} = \frac{\text{Control (A)} - \text{Sample (A)}}{\text{Control (A)}} \times 100$$

Where, A is the absorbance of the sample and control.

**Anti-diabetic test (α-Glucosidase inhibition assay)**

The α-glucosidase inhibitory activity was assessed by the standard method (Dong *et. al.*, 2012) with slight modifications.

***Preparation of Reagent***

1. Preparation of Buffer solution

   7.393 gm of Potassium dihydrogen orthophosphate and 7.956 gm of Dipotassium hydrogen orthophosphate were weighted and dissolved in 1 litre of distilled water. Here, pH of the buffer was maintained 6.8 by adding mono-potassium or di-potassium phosphate.

2. Preparation of Acarbose and plant extract

   100 µg/ml concentration of Acarbose was prepared by mixing 1 mg Acarbose in 10 ml phosphate buffer solution. Different concentrations of the Acarbose solution (20, 10, 5, 2.5, 1.25 µg/ml) was prepared by diluting the stock solution of Acarbose. 1000 µg/ml of plant extract was prepared in buffer solution and was diluted to different concentrations (1000, 500, 250, 125, 62.5, 31.5 µg/ml) using buffer solution.

***Procedure***

The α-glucosidase inhibition of the plant extract was analyzed by using substrate PNPG. 20 µl plant extract and Acarbose of different concentrations (1000, 500, 250, 250,125, 62.5, 31.25 µg/ml) were loaded triplicate in microplate. 10 µl of the α-glucosidase enzyme was loaded in each plate. Similarly, 130 µl was incubated for 15 minutes at 37°C. After initial incubation, initial reading of the plate was taken at wavelength 405 nm. After incubating the solution, 20 µl of 5 mM PNPG was added to each well on microplate. Then, the reaction mixture was incubated for 15 minutes at 37°C. After final incubation, 20 µl of $Na_2CO_3$ was loaded to each well. Here, yellow colour of the para-Nitrobenzene was determined at 410 nm by using a microplate reader (Epoch2, BioTek, Instruments, Inc., USA). The percentage of α-glucosidase activity was calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control (A) - Sample (A)}}{\text{Control (A)}} \times 100$$

Where, A is the absorbance of the sample and control.

## Acute Oral Toxicity Test

The guidelines for Testing of Chemicals, Acute Oral Toxicity, Acute Toxic Class Method 423 of the Organization for Economic Cooperation and Development (OECD) was used. A total of 18 female swissabino mice (6 mice/group), were randomly selected and marked for individual identification. All the animals were subjected to 4 hours of fasting prior to the treatment. The test groups included a control group (10 ml/kg distilled water) and two other treatment groups viz; Group I and Group II for dose 300 mg/kg and 2000 mg/kg body weight of extracts respectively. The animals were observed for 1 hour after treatment, and then intermittently for 4 hours, and thereafter the mice were further observed for up to 14 days following treatment. Clinical signs such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharge from eyes and ears, noisy breathing and the number of deaths in each treated groups were monitored carefully. Body weight, food and water intake were measured on 1$^{st}$, 7$^{th}$ and 14$^{th}$ day.

## Results: Phytochemistry, Antioxidant, Antidiabetic activities and Toxicity

*Preliminary qualitative phytochemical analysis*

The result of preliminary qualitative phytochemical analysis is given in table 1.

**Table 1.** Result of preliminary qualitative phytochemical analysis.

| S.No. | Parameters | Phytochemical Test | Leaf | Bark |
|---|---|---|---|---|
| | Volatile oils | Spot test/Residue test | - | - |
| | Alkaloids | Mayer's Regent test, | + | ++ |
| | Flavonoid | Shinoda test | +++ | +++ |
| | | Alkaline Reagent test | | |
| | Steroids | Steroid test | + | +++ |
| | Terpenoids | Terpenoids test | + | ++ |
| | Glycosides | Salkowski's test | +++ | +++ |
| | Phenols | $FeCl_3$ test | +++ | +++ |
| | Saponins | Froth/ Foam test | - | - |
| | Protein | Ninhydrin test | - | - |
| | Carbohydrate | Molish test | ++ | - |

**Note: Result + means presence in traces amount, ++ means presence in moderate amount, +++ means presence in adequate amount and – means absence.*

## Determination of Total Phenolic Content/Total Flavnoid Content, Antioxidant, Antidiabetic and Acute Oral Toxicity

The TPC, TFC, Antioxidant, Antidiabetic activity and Oral toxicity values of the leaf and bark extract is presented in table 2.

**Table 2.** Result of phytochemical tests for TPC, TFC, antioxidant, antidiabetic activity and oral toxicity.

| S. No. | Plant (part used) | TPC (mgGAE/g) | TFC (mgQE/g) | Antioxidant $IC_{50}$ (µg/mL) | Antidiabetic % inhibition (µg/mL) | Oral toxicity $LD_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|
| 1. | **P. marsupium** (leaf) | 1114.76 | 5.73 | 15.21222 | 98.73 | >2000 |
| 2. | **P. marsupium** (bark) | 596.7 | 4.61 | 43.71872 | 98.32 | >2000 |

Leaf extract showed comparatively higher total phenolic and total flavonoid contents than that of bark. The $IC_{50}$ value of Quercetin for antioxidant assay was found to be 3.33. However, the antioxidant potential was higher in bark extract compared to the leaves extract. Antidiabetic property was nearly equal in both leaf and bark extracts. Similarly, toxicity values (the median lethal dose ($LD_{50}$) of active principle of extract) revealed that the doses of the $LD_{50}$ were >2000 mg/kg, for both the bark and leaf extract indicating non-toxicity at all.

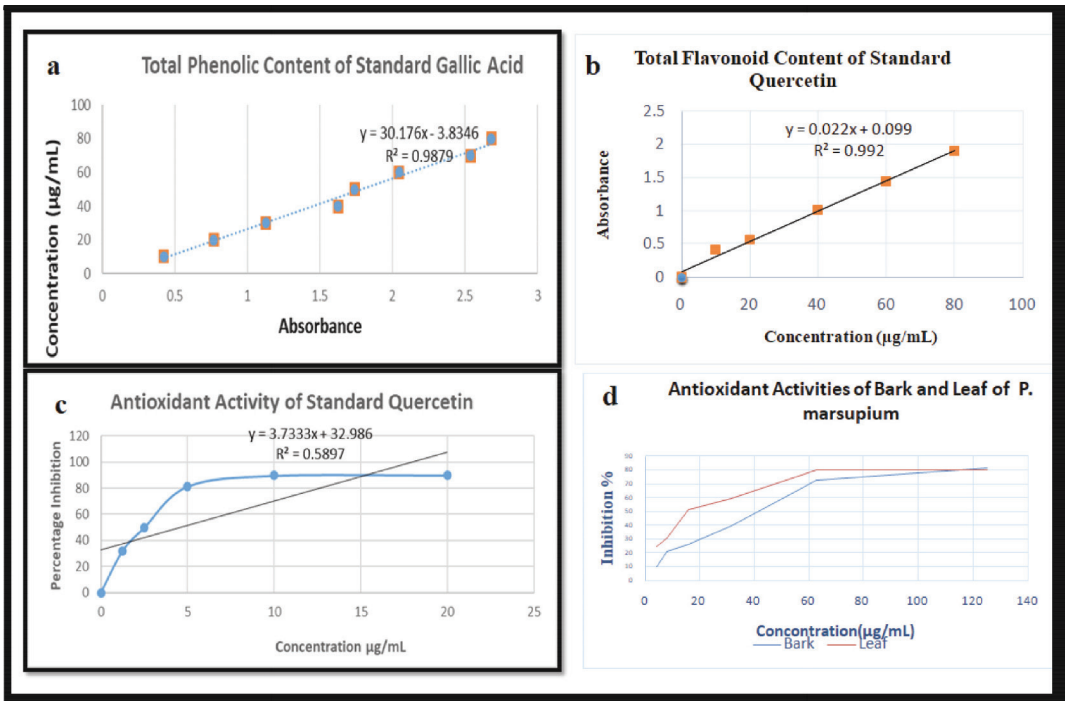


**Figure 1: a.** Total Phenolic Content of Standard Gallic Acid; **b.** Total Flavonoid content (TFC) of Standard Gallic Acid; **c.** Antioxidant Activity of Standard Quercetin against DPPH; and **d.** Antioxidant Activity of Bark and Leaf of *P. marsupium*.

The overall laboratory tests suggested that *P. marsupium* is rich in phytochemicals and possesses good antioxidant and antidiabetic properties. It possesses high antioxidant activity that might be proposed for impeding toxic oxidation in nutraceuticals or drugs for the treatment of coronary diseases. It has strong inhibitory activity against α-glucosidase and can be used to formulate antidiabetic drugs. Non-toxicity of the extracts indicates its applicability as a drug. Further investigations regarding the isolation and identification of responsible antioxidants and antidiabetic components and their mechanism of action is necessary. This will enable us to better understand their ability to control diseases and could have a significant impact on the quality of life.

# CHAPTER - 10
# ANTI-MICROBIAL ACTIVITY

**Pramesh Bahadur Lakhey and Sachita Joshi**

Various parts of the *Pterocarpus marsupium* tree have been used as traditional ayurvedic medicine. The medicinal utilities have been described, especially for leaf, fruit and bark. *In vitro* testing of antimicrobial activity of extracts from different parts of this plant has been carried out by different authors. Bhat *et al.* (2014) reported antimicrobial activity of heartwood extract. Similarly, Pant *et al.* (2017) evaluated antimicrobial activity of stem wood. Manne *et al.* (2020) tested chitosan synthesized nanoparticles from the heartwood extract, for its antimicrobial property. Antimicrobial studies on its extracts have also been done by other authors including Deepa *et al.* (2014), Gayathri and Kannabiran (2021) and Ramya *et al.* (2008).

## Materials and Methods

### *Sample Source*

Approximately, one-kilogram fresh weight of leaf-bearing twigs and bark samples of *P. marsupium* were collected from Dhakeri Botanical Garden, Banke. After the collection of samples, herbarium specimens were prepared and housed at National Herbarium and Plant Laboratories, Godawari, Lalitpur.

### *Preparation of extract*

The samples were washed in tap water to remove adhering soil particles. The leaves were manually plucked from twigs. The twig and bark samples were cut into small pieces using pruning shears. The leaves, pieces of twigs and bark were then spread into a thin layer and shade dried. The dried samples were separately ground into coarse powder in a mixer-grinder.

Extraction was done from the powdered samples by modified maceration process (steady-state extraction) as described by Singh (2008) in three phases using Hexane, Ethyl acetate and Methanol as menstruum for each phase. For the first extraction phase, 15 gm of each sample was placed in a 250 ml conical flask and 150 ml Hexane (ten times the weight of the sample) was added to it. The conical flasks were agitated at 150 rpm in a rotary flask shaker for the extraction duration of a week in a continuous cycle of 60 seconds agitation followed by 30 seconds rest. The extraction was done in four replicates. After seven days, the mixture so formed was allowed to stand for 24 hours and the supernatant was strained off into a separate vessel. The solid residue or marc was transferred into three layers of muslin cloth and was pressed to release as much occluded solution as possible. The strained and

occluded liquids were mixed and was allowed to stand for 24 hours. The mixture was further clarified by filtering through Whatman No.1 filter paper under vacuum using a Buchner funnel. The miscella, so obtained, was concentrated in a rotary vacuum evaporator. The concentrated miscella was further dried on a concentric ring water bath to yield a dry extract.

Marc from the first phase of extraction was shade dried for 48 hours to remove residual solvent and was used for the second phase of extraction using Ethyl acetate as menstruum. The same process was repeated in the third extraction phase with the marc from second phase using Methanol as menstruum. The resulting extracts from the three extraction phases were separately weighted, collected in a McCartney bottles, labelled and stored in a refrigerator at 2-8ºC until further use.

The yield of different extracts was calculated by using the given formula:

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of dry powder taken}} \times 100\%$$

### *Screening of extracts for Antimicrobial Activity*

### Test organisms

The extracts were tested for antimicrobial activity against eleven bacterial strains/ isolates belonging to 10 bacterial species and 2 fungal strains belonging to two fungal species which have been listed in table 1 .

**Table 1.** List of bacterial and fungal strains used as test organisms.

| S.No. | Name of species | Reference No. | Strains | Source |
|---|---|---|---|---|
| 1 | *Bacillus subtilis* | ATCC-6051 | Gram positive | Microbiologics, 200 Cooper Avenue North, St, Cloud MN56303 |
| 2 | *Escherichia coli* | ATCC-8739 | Gram negative | |
| 3 | *Enterococcus faecalis* | ATCC-29212 | Gram positive | |
| 4 | *Klebsiella pneumoniae* | ATCC-700603 | Gram negative | |
| 5 | *Proteus vulgaris* | ATCC-6380 | Gram negative | |
| 6 | *Pseudomonas aeruginosa* | ATCC-9027 | Gram negative | |
| 7 | *Salmonella enterica enterica* Typhi | Clinical Isolate | Gram negative | TU Teaching Hospital, Maharajgunj, Kathmandu |
| 8 | *Shigella dysenteriae* | Clinical Isolate | Gram negative | |
| 9 | *Staphylococcus aureus* | ATCC 6538P | Gram positive | Microbiologics, 200 Cooper Avenue North, St, Cloud MN56303 |

| S.No. | Name of species | Reference No. | Strains | Source |
|---|---|---|---|---|
| 10 | Methicillin resistant *S. aureus* (MRSA) | Clinical Isolate | Gram positive | Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu |
| 11 | *Staphylococcus epidermidis* | ATCC 1228 | Gram positive | Microbiologics, 200 Cooper Avenue North, St, Cloud MN56303 |
| 12 | *Candida albicans* | ATCC.2091 | Fungi | |
| 13 | *Saccharomyces cerevisae* | ATCC.18824 | Fungi | |

**Preliminary screening for antimicrobial activity**

The extracts were screened for antimicrobial activities following modified Agar well diffusion method, described by Nathan *et al.* (1978) and Holder and Boyce (1994). Clinical and Laboratory Standards Institute (2012) and National Committee for Clinical Laboratory Standards (2004) were also referred to.

Test solution of each extract of 100 $mg.ml^{-1}$ concentrations was prepared in dimethyl sulphoxide (DMSO). Inoculation suspension of each of the test organism with turbidity equivalent to 0.5 McFarland Nephelometric Standard was prepared in sterilized normal saline using 18 to 24 hours culture by direct colony suspension method. A sterile cotton swab was used to evenly distribute bacterial or fungal culture drawn from the respective inoculums over the appropriate medium, viz. Muller-Hinton Agar (MHA) for bacteria and Muller-Hinton Agar with Glucose and Methylene Blue (MHA.GMB) for fungi (Clinical and Laboratory Standards Institute, 2012; National Committee for Clinical Laboratory Standards, 2004). The inoculated plates were left for maximum 15 minutes to allow absorption of excess surface moisture. Using sterilized cork borer, agar wells of 6 mm diameter were made in the inoculated plates, 4 to 5 wells per plate. Micropipettes were used to place 50 µl of the test solution into each well. DMSO (50 µl) was also added to one of the wells of each plate as negative control. Antibiotic sensitivity discs were used as positive control for bacterial species; Ciprofloxacin 5 µg for *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enterica enterica* Typhi and *Shigella dysenteriae*; Cefoxitin 30 µg and Azithromycin 30 µg for *Staphylococcus aureus*, Methicillin resistant *S. aureus* (MRSA) and *S. epidermidis*. The plates were placed in obverse position until the test solution was absorbed into agar and the wells appeared dry. The plates were then incubated in inverted position at 35±2°C for 18 to 24 hours for all test organisms except *Saccharomyces cerevisiae* which was incubated at 25±2°C for 24 to 48 hours.

After the incubation period, the diameters of zones of inhibition (ZOIs), interpreted as the clear areas around the agar wells, were measured using a digital caliper. The test for antimicrobial activity was done in five replicates for each of the test species.

**Determination of minimum microbicidal concentration**

Broth macro-dilution method described by National Committee for Clinical Laboratory Standards (1999) was followed to determine minimum microbicidal concentration (MMC) of the test extracts. Twelve vials, each containing 1 ml of Muller Hinton Broth for bacteria and Sabauraud Dextrose Broth of fungi were prepared and labelled from 0 to 11. In no. 0 vial, the media was removed and only 1 ml test solution of 100 $mg.ml^{-1}$ concentrations was placed. In no. 1 vial, 1 ml of the test solution was added to 1 ml of the medium and mixed evenly by vortexing to obtain a solution of concentration 50 $mg.ml^{-1}$; 1 ml of the 50 $mg.ml^{-1}$ solution in no. 1 vial was transferred to no. 2 vial and was mixed evenly to obtain a solution of concentration 25 $mg.ml^{-1}$. This process was repeated up to vial no. 10 to obtain solutions of concentrations 12.5 $mg.ml^{-1}$, 6.25 $mg.ml^{-1}$, 3.125 $mg.ml^{-1}$, 1.5625 $mg.ml^{-1}$, 0.78125 $mg.ml^{-1}$, 0.390625 $mg.ml^{-1}$, 0.1953125 $mg.ml^{-1}$ and 0.09765625422 $mg.ml^{-1}$. Vial no. 11 was left with medium only. Each vial was inoculated with 20 µl of the cell suspension of the organisms with turbidity equivalent to 0.5 McFarland Nephelometric Standard and were incubated at 35±2°C for 18 to 24 hours for all test species except *Saccharomyces cerevisiae* which was incubated at 25±2°C for 24 to 48 hours. The MIC of the samples were visually determined as the lowest concentration that did not show any growth as indicated by turbidity in comparison to the uninoculated medium. For the determination of MMC, the vials were sub-cultured on nutrient agar (NA) for bacterial species and Sabauraud dextrose agar (SDA) plates for fungal species to determine the viability of the inoculated organisms at different concentrations of the extracts and were incubated at suitable temperature and for suitable durations. MMC was determined as the concentration value between the minimum concentration with no growth and maximum concentration showing growth.

**Statistical analysis**

The charts were generated using Microsoft Excel 2013. The extract yield percentages were compared by ANOVA followed by Tukey HSD test, and ZOI values by paired sample t-test using SPSS 23.0.

**Results: Antimicrobial activity**

The yield of extract from bark, leaves and twig samples of *Pterocarpus marsupium* in Hexane, Ethyl acetate and Methanol differed significantly ($df = 8,27$, $F = 19758.396$, $p = 0.000$, Figure 1). The maximum extract yield was observed in methanol extract

of bark (10.33±0.050%) followed by methanol extract of twigs (9.91±0.057%) while the minimum yield was observed in Hexane and Ethyl acetate extracts of bark (0.34±0.052% and 0.036±0.070% respectively). The high yields of methanol extracts from the leaf, bark and twig samples of *P. marsupium* indicate that these parts are rich in polar compounds. In comparison to bark and twigs, leaves contained more non-polar and mid-polar components as evident from the yield of Hexane and Ethyl acetate extracts of these samples.

During preliminary antimicrobial activity screening by agar well diffusion method, only methanol extracts of bark and twigs showed antimicrobial activity against *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA) and *S. epidermidis*; while methanol extract of leaves showed activity against *S. aureus* and *S. epidermidis* (Plates 1a, 1b and 1c). However, all the tested plant extracts did not show any antimicrobial activity against *Bacillus subtilis, Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica entrica* Typhi*, Shigella dysenteriae*, *Candida albicans* and *Saccharomyces cerevisiae* (Zone of Inhibition (ZOI) = 0 mm). In contrast to the findings of this study, Patil and Gaikwad (2011) have reported antimicrobial activities of bark extract of *P. marsupium* against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoneae*, *Salmonella typhi*, *Proteus mirabilis* and *Micrococcus* sp. They concluded *Staphylococcus aureus* as being most sensitive to the extract. Ramya *et al.* (2008) found hexane, ethyl acetate and methanol extracts of bark and leaves of this plant species as effective against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Streptococcus pyrogens, Escherichia coli, Salmonella typii, Serratia marcescens* and *Pseudomonas aeruginosa* with inhibitory activity being dose dependent. They found ethyl acetate and methanol extracts to be more active towards the organisms tested than hexane extract. Gayathri and Kannabiran (2021) reported antimicrobial activity of aqueous extract of roots of this plant against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Bhat *et al.* (2014) found that the heartwood aqueous extract of *P. marsupium* was effective against *Enterococcus* sp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Deepa *et al*. (2014) reported antimicrobial activity of the ethanol extract of bark of this species against *Bacillus polymyxa*, *Vibrio cholera* and *Candida albicans.*

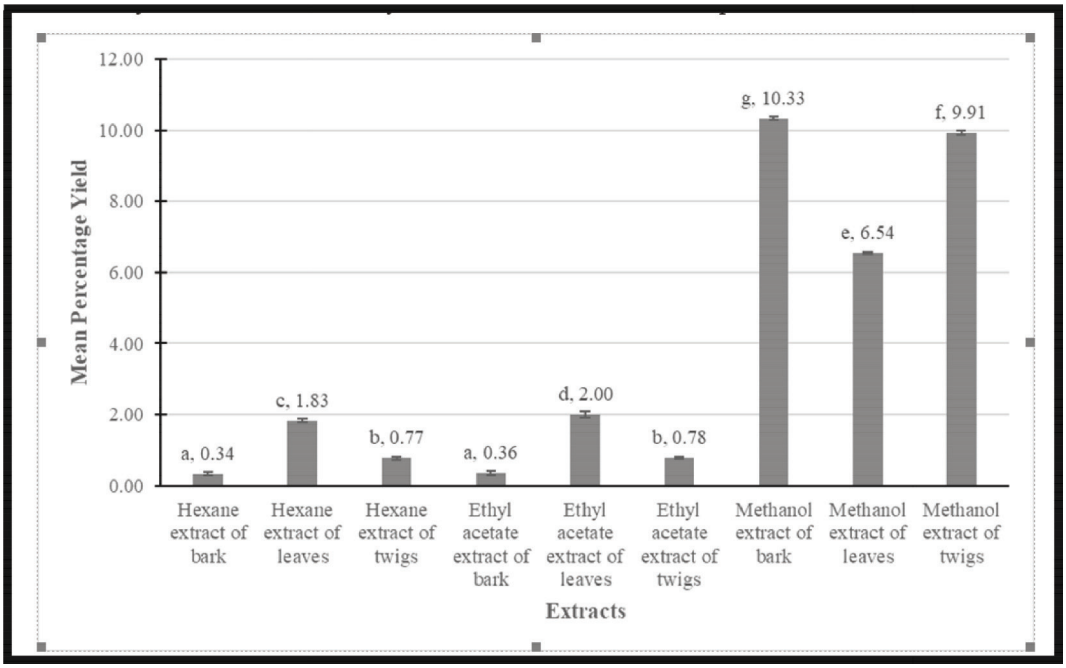


**Figure 1:** Percentage yield of extracts from bark, leaves and twig samples. Different alphabets above the error bars indicate significant difference at 5% level of significance as determined by Tukey's HSD test. The numbers above the error bars are mean yield percentages. The error bars indicate ± 2SEM.

Cefoxitin 30 µg sensitivity disc has been recommended for determining antibiotic resistance (methicillin/oxacillin resistance) in strains/isolates of *S. aureus* (Clinical and Laboratory Standards Institute, 2012). During this study, the sensitivity of MRSA to cefoxitin 30 µg disc, as indicated by ZOI, was found to be significantly less in comparison to that of sensitive *S. aureus* strain ATCC 6538P ($df = 2$, $t = 38.602$, $p = 0.001$, Figure 2, Plates 1a and 1b). On the other hand, there was no significant difference between the antimicrobial activities shown by methanol extract of bark against *S. aureus* ATCC 6538P and MRSA ($df = 4$, $t = 1.075$, $p = 0.343$, Figure 3, Plates 1a and 1b). Similar result was also observed in case of methanol extract of twigs ($df = 4$, $t = 0.480$, $p = 0.657$, Figure 4, Plates 1a and 1b). However, the methanol extract of leaves which showed antimicrobial activity against *S. aureus* ATCC 6538P (ZOI=8.88±0.83 mm) did not show activity against MRSA (ZOI = 0).

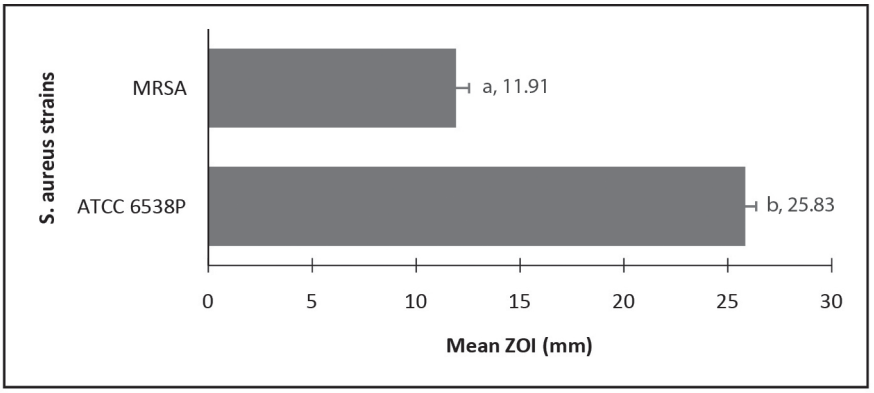


**Figure 2:** ZOIs of cefoxitin 30 µg disc against *S. aureus* strains. Different alphabets to the right of the error bar indicates significant difference between means as determined by paired-sample t test at 1% level of significance. The number to the right of error bar is the mean ZOI. The error bars indicates ± 2SEM.

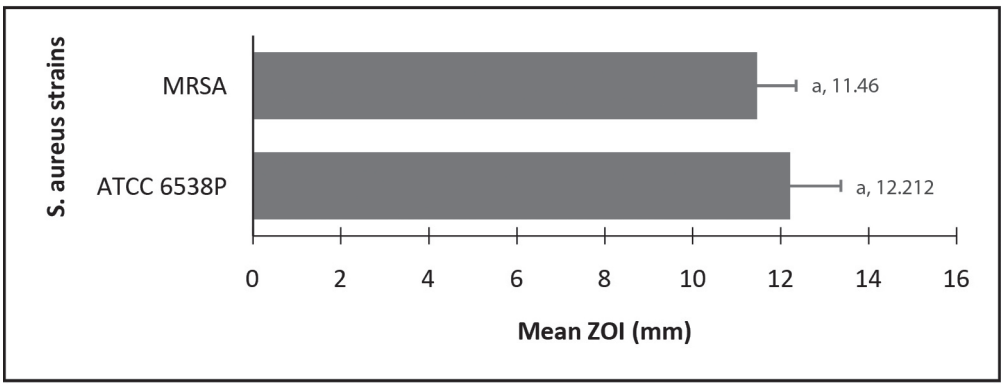


**Figure 3:** ZOIs of methanol extract of bark against *S. aureus* strains. The same alphabet to the right of the error bar indicates no significant difference between mean as determined by paired-sample t test at 1% level of significance. The number to the right of error bar is the mean ZOI. The error bars indicates ± 2SEM.

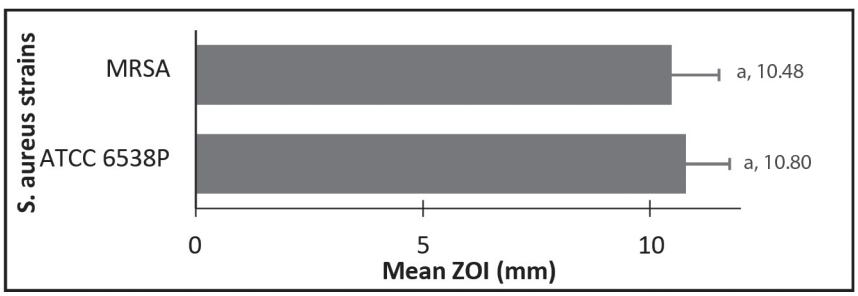


**Figure 4:** ZOIs of methanol extract of twigs against *S. aureus* strains. The same alphabet to the right of the error bar indicates no significant difference between mean as determined by paired-sample t test at 1% level of significance. The number to the right of error bar is the mean value. The number to the right of error bar is the mean ZOI. The error bars indicates ± 2SEM.

Among the extracts tested for minimal microbicidal concentration (MMC), the least MMC of 3.1256.25 mg.ml$^{-1}$ was measured for methanol extract of bark against *S. aureus* ATCC 6539P. The same extract showed higher MMC value between 6.2512.5 mg.ml$^{-1}$ for MRSA. The highest MMC value was observed in case of methanol extract of leaves against *S. aureus* ATCC 6539P (Table 1, Plates 3 and 4).

Gayathri and Kannabiran (2021) found ZOI values of aqueous extract of roots of this tree species between 0.04 to 0.09 mg.ml$^{-1}$ against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The ethanol extract of bark has been shown to have minimum inhibitory concentration of 1.25 mg.ml$^{-1}$ against *Bacillus polymyxa* and *Vibrio cholerae* while inhibition of *Candida albicans* was found at 25 mg.ml$^{-1}$ through broth dilution method (Deepa *et al.,* 2014; Gayathri and Kannabiran, 2021; Katiyar *et al.,* 2016).

**Table 2.** Minimum microbicidal concentrations (MMCs) of the extracts showing antimicrobial activities in preliminary screening.

| S. No. | Extract | Bacterial strain | Minimum microbicidal Concentrations (mg.ml$^{-1}$) |
|---|---|---|---|
| 1 | Methanol extract of Bark | *S. aureus* ATCC 6539P | 3.125-6.25 |
| | | MRSA | 6.25-12.5 |
| | | *S. epidermidis* | 6.25-12.5 |
| 2 | Methanol extract of Leaves | *S. aureus* ATCC 6539P | 25-50 |
| | | MRSA | Not done due since ZOI = 0 |
| | | *S. epidermidis* | Not done due since ZOI = 0 |
| 3 | Methanol extract of twigs | *S. aureus* ATCC 6539P | 6.25-12.5 |
| | | MRSA | 6.25-12.5 |
| | | *S. epidermidis* | 3.125-6.25 |

This study validates the traditional use of "Bijaysal" for medicinal purposes for skin related ailments. It strongly indicates the suitability of methanol extracts of bark and twigs for the treatment of diseases caused by *Staphyloccus aureus* and its antibiotic resistant strain MRSA. The active ingredients in these extracts should be identified and isolated so that the efficiency of this natural herbal medicine can be increased.

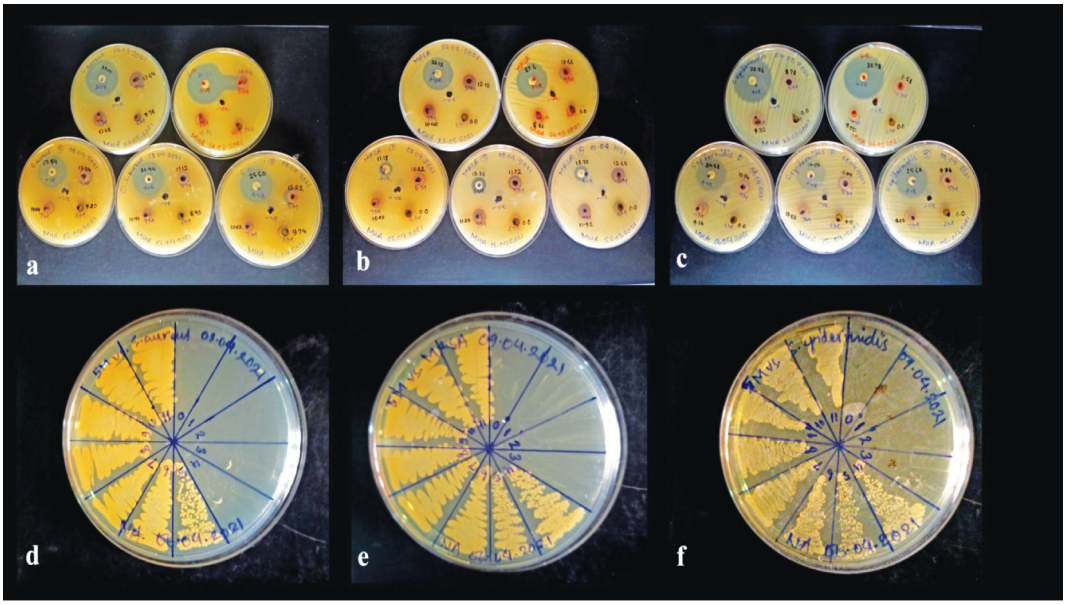


**Plate: 1a.** Preliminary screening of antimicrobial activity of methanolic extracts of *Pterocarpus marsupium* bark, leaves and twigs samples against *Staphylococcus aureus* by agar well diffusion method. The wells on each plate from top right hand side in clockwise direction contains methanolic extract of bark (5M), methanolic extract of leaves (6M), methanolic extract of twigs (7M) and positive control (azithromycin 30 µg disc in the upper two plates, cefoxitin 30 µg in lower 3 plates). The central well contains DMSO as negative control; **b.** Preliminary screening of antimicrobial activity of methanolic extracts of *Pterocarpus marsupium* bark, leaves and twigs samples against methicillin resistant *Staphylococcus aureus* (MRSA) by agar well diffusion method; **c.** Preliminary screening of antimicrobial activity of methanolic extracts of *Pterocarpus marsupium* bark, leaves and twigs samples against *Staphylococcus epidermidis* by agar well diffusion method; **d.** Determination of minimum microbicidal concentration (MMC) of methanolic extract of *Pterocarpus marsupium* bark against *Staphylococcus aureus* by subculturing of two fold inoculated two-fold serial dilution vials in each of the section of petridish labelled 0 to 11; **e.** Determination of minimum microbicidal concentration (MMC) of methanolic extract of *Pterocarpus marsupium* bark against methicillin resistant *Staphylococcus aureus* (MRSA); and **f.** Determination of minimum microbicidal concentration (MMC) of methanolic extract of *Pterocarpus marsupium* bark against *Staphylococcus epidermidis.*

# CHAPTER - 11
# THREATS, CONSERVATION AND TRADE

**Dipesh Pyakurel and Pashupati Nath Koirala**

Ecological resources from forests such as timber, non-timber and medicinal plants play a vital role in the livelihoods of rural communities around the globe. It has been estimated that environmental income accounts for 28% of the total household income in the developing world (Angelsen *et al.*, 2014). The higher dependency may exert direct pressure on the sustainability of species with high demand and price making these species vulnerable. Examples of exploitation of high valued species are seen from Africa (Gaoue and Ticktin, 2008), Nepal (Pyakurel *et al.*, 2019), India (Chauhan *et al.,* 2018), and South America (Cruz-Garcia *et al.,* 2015).

*Pterocarpus marsupium* Roxb. (Bijaysal) is a slow growing tree species with limited geographical distribution from tropical South Asia to Taiwan (Barstow, 2017). In Nepal, it is naturally distributed in foothills of Chure (Siwalik) in Kanchanpur, Kailali, Bardiya, Banke, Kapilbastu, Rupandehi, Palpa and Nawalparasi districts within 100 to 500 m. The population, however, is very sparsely distributed (DoF, 2018).

This chapter identifies the threats, analyzes the conservation status, and discusses about the trade of *P. marsupium* in Nepal. Both electronic and online database e.g., Google Scholar, PubMed, Research gate, Scopus, Science Direct were conducted by typing key words like *Pterocarpus marsupium*, trade, threats, conservation. Online searches were conducted for unpublished reports. Likewise, other sources like books, reports, thesis, and newspaper articles were also referred to collect the information. Entrepreneurs and traders from Sudurpaschim province were individually contacted to generate trade related information regarding per unit price of different items and annual turnovers.

## Existing threats and mitigation measures

Low germination and slow growth, high grazing pressure, fodder collection, and harvesting of wood (to manufacture glass, cups, bowls, lota[1], amkhora[2] and agricultural equipment) and *Kino* gum for economical and medicinal use are the major threats to *P. marsupium*, due to which the global population is declining (Wilkins, 1991).

Bijaysal is sparsely distributed even in its area of natural occurrence. Only 395 mature trees were recorded from 12 Community Forests (CF) of Kanchanpur district in

1 *Lota: A traditional Nepalese utensil made by brass and used to carry small quantity of water.*
2 *Amkhora: A traditional Nepalese utensil made by brass and with a long spout/pourer; used to drink water.*

2014. The average number of mature trees was 9- 10 per hectare (Pyakurel and Oli, 2014). Likewise, 500 mature trees were recorded from Saraswati CF, Buddhabhumi Municipality-10 of Kapilbastu district (DoF, 2018), showing the sparse distribution pattern of *P. marsupium* in Nepal. Aggravated to that, it is a slow growing species taking at least 15 years to mature (Vikaspedia, 2021).

The tree has multiple uses ranging from medicinal, economic and ecological (Pyakurel and Oli, 2014; Barstow, 2017; DoF, 2018). People rely on wild stands of Bijaysal for medicinal and economical uses. The Kino gums are extracted from the trunk of tree by making scars. The ecological implications of such practices are not well documented. The trees are cut and fell — despite protected by the Government of Nepal under Forest Regulation 1995, for the preparation of bijaysal cups, glasses, bowls, and other items which are praised in Nepalese and Indian communities for its health benefit and as a decorative item. Only dead and fallen trees are allowed to collect by law but high demand of items made from its wood causes the looping of standing trees, thereby maximizing the risk of disappearance from the wild. This scenario shows that ban on products with huge commercial value may not be effective. Alternatively, governments e.g., Division Forest Offices of western Tarai should start raising seedlings and saplings of *P. marsupium* in government nurseries and promote private nurseries to do so. Successful raising and plantation has been observed in Rupandehi as a research plot established by Forest Research and Training Centre (the then Department of Forest Research and Survey). Sparse plantation has been initiated by the government in Bardiya (by Plant Research Centre, Kailali) and other districts too. Private nurseries can be promoted to raise seedlings of *P. marsupium* and later cultivation in private lands can be initiated.

Livestock, along with deer and other wild animals feed on seedlings and saplings of *P. marsupium* as leaves are highly palatable. Communities also cut small branches for fodder, thereby rendering its natural regeneration. However, sensing its ecological and economical importance, some community forests have prohibited the open grazing by promoting stall feeding of cattle, penalty on open grazing in CFs (Pyakurel and Oli, 2014). Such successful practice adopted by Betkot CF, Baijanath CF, Mahakali CF and Ramnagar CF in Kanchanpur districts should be replicated in other CFs and natural forests where *P. marsupium* is naturally distributed.

**Conservation status and Initiatives**

Natural stands of *Pterocarpus marsupium* are said to be 'fast disappearing' in the natural habitat (Anis *et al.,* 2005). The major threat to its natural population is low germination percentage and cutting and felling for various purposes. The natural regeneration takes place by means of seed, but the germination percentage is only 30% and even low (Kalimuthu and Lakshaman, 1995). Due to overexploitation of

the tree for its various useful application coupled with low germination and very limited distribution, *P. marsupium* is banned for felling, transportation, and export by Government of Nepal under the Forest Regulation of 1995 (amended in 2001). It falls in 'Near Threatened' category of International Union for Conservation of Nature (IUCN) Red List because of the threats present to the species, decreasing population and declining areas of occurrence (Barstow, 2017).

There are, however, few conservation initiatives taken by the government. The Bijaysal Conservation and Action Plan, Nepal 2018-2022 was prepared by Department of Forests for its long-term conservation, increasing the population in the wild, and to strengthen the multi-stakeholder participation. Few other initiatives taken for conservation of *Pterocarpus marsupium* are: (i) *in-situ* and *ex-situ* conservation were initiated in few western Tarai districts (e.g., in Kanchanpur, Kapilbastu); (ii) a breeding orchard was established by Department of Forest Research and Survey in Jogikuti, Butwal; (iii) seedlings raised in nursery in Dhangadi (operated by Department of Plant Resources and Division Forest Office) and Kanchanpur (Division Forest Office) including other CF forest nurseries; and (iv) resource assessment of *P. marsupium* was conducted by District Forest Office in twelve community forests of Kanchanpur district (DoF, 2018) and due to conservation initiative adopted by CFs, the number of *P. marsupium* is increasing (personal communication). Raising seedling and plantation have been initiated in other districts too.

A few private nurseries are also raising seedlings of *P. marsupium* (e.g., in Bardiya district) but the objective taken by Bijaysal Conservation and Action Plan, Nepal 2018-2022 in increasing the wild population by 15%, is not fulfilled— showing the need of more coordinated work among the government, communities and private sectors.

**Figure 1:** Bijaysal breeding seed orchard at Krishnapur, Kanchanpur district. (**Photos:** a. Pashupati Nath Koirala; and b. Pratikshya Chalise).

## Trade

Owing to its multiple medicinal use specially to treat gastro-intestinal disorder and diabetes, *Pterocarpus marsupium* has been used in traditional, ayurvedic for many centuries (Abirami *et al.,* 2012; RPRC, 2014). As a result, items made from *P. marsupium* have high demand, resulting into higher market price. Given below is the range of products made from wood of *P. marsupium* in 2020, along with their price.

**Table 3.** Products and prices of items made from Bijaysal in 2020.

| S. No. | Product Name | Selling price (NPR) |
|---|---|---|
| 1 | Amkhora | 700 – 800 |
| 2 | Lota | 800 |
| 3 | Bowl | 700 |
| 4 | Cup | 400 – 500 |
| 5 | Glass | 600 – 700 |
| 6 | Chips/ Piece | 50 – 100 |

Out of those products, cups have high market demand (Figure 2), followed by glass. Amkhora, Lota and Bowls are made in less quantity but per unit price is high. Moreover, small chips/pieces of wood also have market value. In Nepal, there are only a handful number of persons who are engaged in manufacturing these items. A direct consultation with two entrepreneurs disclosed that only 4- 6 persons are skilful to manufacture items made from *P. marsupium,* but now because of the lower availability of the woods from the community forests, only two registered enterprises are in operation: one is operating since 2000 and second one from 2015, both are located in Kanchanpur district. Their individual annual turnover ranged between NPR 4- 5 hundred thousand. Apart from these two, a very handful number of unregistered enterprises operate even in a smaller scale. Their cumulative annual trade does exceed NPR 0.5 million. Thus, it can be conservatively estimated that the annual trade of items made from *P. marsupium* in and from Nepal is around NPR 1- 1.5 million.

**Figure 2.** Cups made from Bijaysal wood. These cups have high demand in Nepal and India. (**Photos:** a. Dipesh Pyakurel; and b. Pashupati Nath Koirala).

Due to the rising demand of items made from *Pterocarpus marsupium*, cultivation initiative in private and community forests, as mentioned earlier will ensure the supply of the raw material in a long run. Once the supply is ensured, new micro-entrepreneurs will start manufacturing and existing entrepreneurs can upscale their production, thereby contributing to household and local economy.

Owing to the multiple health benefits of *Pterocarpus marsupium*, the demand will rise and to fulfil the growing demand, entrepreneurs will collect *P. marsupium* trees, one way or other. Thus, a cultivation initiative is felt necessary which serves multiple purposes: (i) ensuring the sustainability of trees in nature, (ii) increasing the population of *P. marsupium* in Nepal and (iii) ensuring the sustainable supply of wood to entrepreneurs in a longer run. Private nurseries should be promoted, along with the nurseries managed by the government. The successful practice of prohibiting open grazing in community forests should be replicated in other CFs and national forests to promote natural regeneration of *Pterocarpus marsupium*.

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# APPENDICES

**Appendix 1.** List of Herbarium specimens examined.

**Western Nepal:**

Baghphanta, Kanchanpur district, Western Nepal, 140m; 1981-10-02 AD; Kattel, L.P., 747; (KATH016545).

Dudhiya Khanta, Kanchanpur district, Western Nepal, 160m; 1980-11-23 AD; Kattel, L.P. and Malla, K.J., 50; (KATH015263).

Gaugi, Mahendranagar, Kanchanpur district, Western Nepal, 300m; 1999-10-22 AD; Bhatta, G.D. and Kurmi, P.P., 1006; (KATH070856).

Kanchanpur district, Western Nepal; 2036- Poush; Bhattacharya., (KATH070860).

Daizi, Kanchanpur district, Western Nepal, 160m; 2049-7-27 BS; Joshi, C.M. and Rijal, H.L., 470/49; (KATH070876).

Malakheti, Kailali district, Western Nepal; 2077-7-15 BS; Chalise, P., Paneru, Y.R. and Bhatta, P., K0020.

Dewaria Botanical Garden, Kailali district, Western Nepal; 2077-7-13 BS; Chalise, P. and Paneru, Y.R., K0014- Planted tree.

Dhakeri Botanical Garden, Banke district, Western Nepal; 2021-01-05 AD; Bhatt, G.D. and Acharya, Y. 77906- Planted tree; (KATH086318).

Dhakeri Botanical Garden, Banke district, Western Nepal; 2021-01-05 AD; Bhatt, G.D. and Acharya, Y. 77907- Planted tree; (KATH086322).

Samaiji Community Forest, Krishnapur-4, Kanchanpur district, Western Nepal, 207m; 2020-12-26; Bhatt, G.D. and Karkee, D., 77819; (KATH086302).

Samaiji Community Forest, Krishnapur-4, Kanchanpur district, Western Nepal, 207m; 2020-12-26; Bhatt, G.D. and Karkee, D., 77819; (KATH086302).

Samaiji Community Forest, Krishnapur-4, Kanchanpur district, Western Nepal, 207m; 2020-12-26; Bhatt, G.D. and Karkee, D., 77820; (KATH086305).

Samaiji Community Forest, Krishnapur-4, Kanchanpur district, Western Nepal, 207m; 2020-12-26; Bhatt, G.D. and Karkee, D., 77821; (KATH086307).

**Central Nepal:**

Bulakiya, Kapilbastu district, Central Nepal, 200m; 1996-3-1 AD; Kurmi, P.P., 10084; (KATH070868).

Butwal, Rupandehi district, Central Nepal, 200m; 2062-9-3 BS; Adhikari, M.K., Joshi, L., Manandhar, V. and Kurmi, P.P., 17; (KATH070861).

Champapur, Kapilbastu district, Central Nepal, 200m; 1992-11-3 AD; Kurmi, P.P., KB451; (KATH070862).

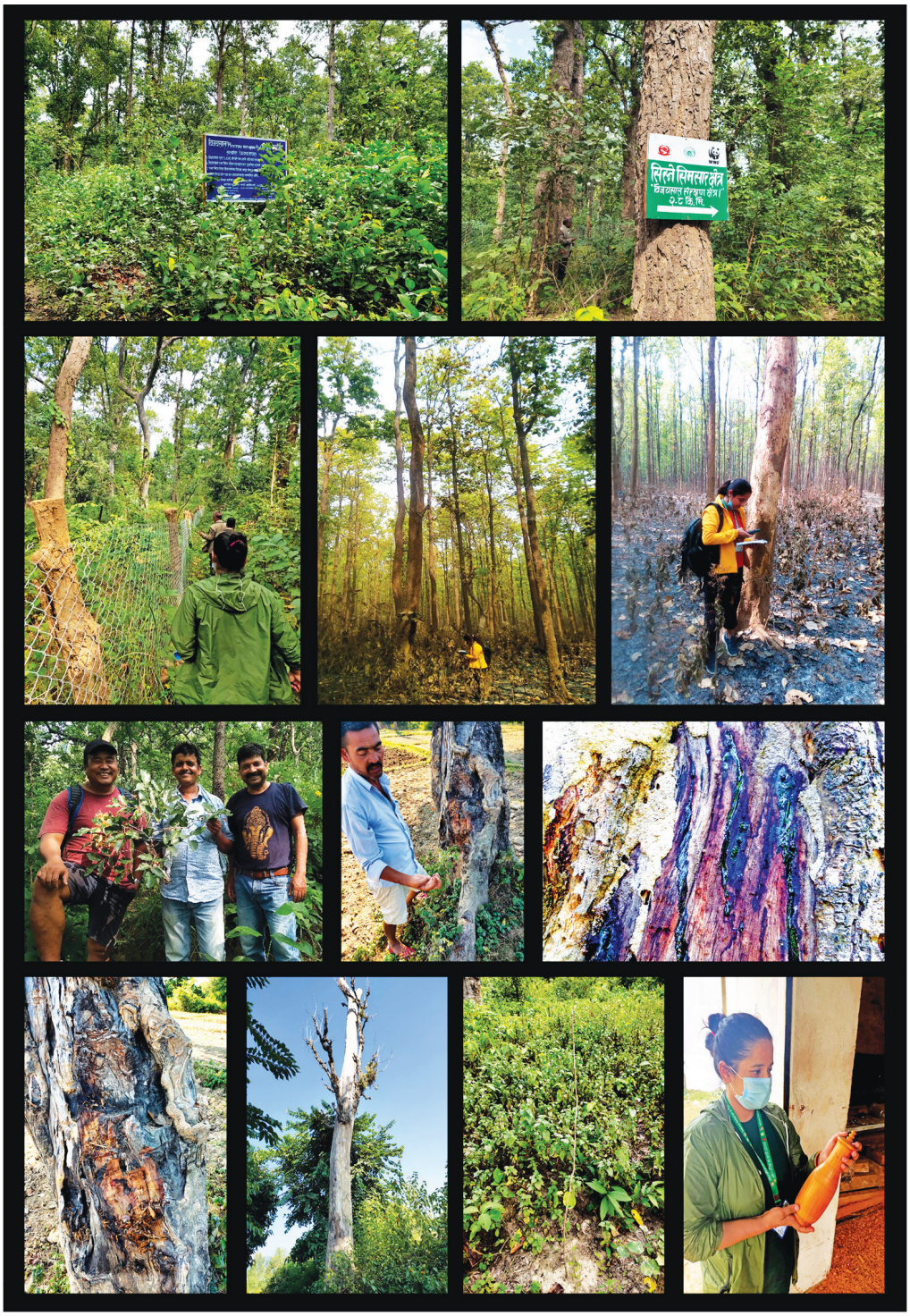
Near Banaganga Rangepost, Kapilbastu district, Central Nepal, 180m; 1998-4-2 AD; Kurmi, P.P. and Bhatta, G.D., 1003; (KATH070871).

Charpala CF, Tamnagar, Rupandehi district, Central Nepal, 2076-7-8 BS; Chalise, P., Paneru, Y.R. and Chalise, G.S., 76K032.

**Eastern Nepal:**

Bhadrapur, Jhapa district, Eastern Nepal, 100m; 2003-1-26 AD; Thapa, N., Bhatta, G.D. and Khatri, S., 2070; (KATH070878)- Planted tree.

**Appendix 2.** Some photographs collected during field observation, data collection, locality tracing, and sample collection of Bijaysal.



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