Medicinal value and microbial VAM incidence analysis of *Bischofia javanica* Blume in Sikkim Himalaya, India

Lepcha L.\textsuperscript{a}, Roy S.G.\textsuperscript{b}, Basistha B.C.\textsuperscript{a}, Sharma, N.P.\textsuperscript{a}, Subba K.B.\textsuperscript{a}, Gurung R.\textsuperscript{a}

\textsuperscript{a} Sikkim State Council of Science & Technology, Development Area, Gangtok-737 101, Sikkim, India

\textsuperscript{b} Department of Botany, West Bengal State University, Barasat, Kolkata, West Bengal, India

**Article history:**
Received: 28 August, 2013
Accepted: 30 August, 2013
Available online: 19 November, 2013

**Keywords:**
Vesicular Arbascular Mycorrhiza (VAM), Geographical Information System (GIS), Medicinal uses, DNA-topoisomerases (Deoxyribose Nucleic Acid), Rhizosphere, Lepcha

**Corresponding Author:**
Lepcha L.*
Information Officer
Email: laydsimick@gmail.com
Phone: +919434191983

Roy S.G.
Reader
Email: sanjoy.guharoy@gmail.com
Phone: +919331019471

Basistha B.C.
Additional Director
Email: Shobihar_99@yahoo.com
Phone: +919434109894

Sharma N.P.
Assistant Scientific Officer
Email: naresthvs@gmail.com
Phone: +919434357738

Subba K.B.
Assistant Scientific Officer
Email: kbsubba_skm@yahoo.co.uk
Phone: +919733003955

**Abstract**
*Bischofia javanica* Blume is one of the valuable tree species of Sikkim Himalaya. The fruit of *Bischofia javanica* is well known edible fruit among the localities of the Himalaya. The tree species has formed an important medicinal significance for curing various diseases like sores, toothache and some eye diseases. With the increasing modern world the physical abundance and medicinal usability and values of *Bischofia* species is under tremendous threat. The main objective of the study was to prepare a database on medicinal uses and to understand VAM ecological system of *Bischofia javanica* in Sikkim Himalaya by assuming Rhizospheric analysis. The distribution pattern and function of Vesicular Arbascular Mycorrhiza presence in the rhizosphere of the *Bischofia javanica* was explored in the study, which support the *Bischofia* species to perform nutritional mechanism and sustain healthy growth in the region. The study also helps to find out the recent status of the species and call on for its value and conservation aspects in the Himalaya.

**Citation:**
Organization has already recommended medicinal plant research warrant attention (WHO, 1980). World Health Organization (WHO, 1996) has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where we lack safe modern drugs. To avail those traditional medicines into pharmacological research and manufacture, depth and fundamental knowledge of ethno medicines are very crucial to understand. The studies on species inhabitants, its morphology, parts and uses help to categorize and simulate the species in the floral kingdom. The micro floral studies will help to understand the microbial mechanism in conducting valuable bacterial activities taking place between rhizomes and bacteria.

The Himalayan state Sikkim lies between 27° 46’ N Latitude and 88° 0’5” - 88°55’25” East Longitude in eastern Himalaya below the world’s third highest Mt. Kanchandzonga (8598m). Sikkim, India is considered to be one of the biodiversity hotspot in the world. It is engulfed with dense forest vegetation, with most of the plant reflecting medicinal recognition. Along this dense vegetation valley, *Bischofia javanica* has occupied its special credibility in medicinal aspects. Sikkim Himalaya is a rich repository of medicinal plants that has been nurturing several distinct ethnomedicinal systems through ages (Pal and Palit, 2011).

However, the mode of prevalence of *Bischofia species* has become a matter of great concern in the state. The density entertained by the species in pre-decade is showing miserable density as compared with the latest census of the species.

2. Review of Literature

Previous studies on *Bischofia javanica* have already shown some interesting findings as a source of medicines and anti-bioactivity inhibitors. In the year 2005 in a published journal of Chemistry and Biodiversity, Japanese researchers Shun -ichi –wada and Reiko Tanaka, has reported upon the isolation of compounds betulinic acid and its derivatives betulonic acid, 3β-O-(Z)- coumaroyl betulinic acid and 3β-O-(E)-coumaroyl betulinic acid, after undergoing an extraction process from the bark of *Bischofia javanica*. These compounds are found to be the catalytic inhibitor of Topo-II enzyme activities. The Topo-II (DNA topoisomerases) is the enzyme which is used as target enzyme for anticancer chemotherapeutic drug development.

Traditionally, the bark is used for the treatment of tuberculosis, stomach ulcer, mouth ulcer, and athlete’s foot (Nayar and Chopra, 1970). As described in a published title of *Bischofia javanica*, Bishop wood by “Invasive Plants of Asian Origin Established in the US and Their Natural Enemies; the bark of *Bischofia javanica* is also considered as source of red dye, the fruits are used in winemaking, containing 30-54 percent oil, the edible seeds are used as a source of lubricant. Newly formed Indian state Uttarakhand has emarked *Bischofia javanica* as one of the major source of black dye (Gaur, 2008).

The root of *Bischofia javanica* has medicinal uses (Li Bingtao, 1994). Leaves are used in the treatment of stomachache and the leaf juice for cancerous wounds (Von Reis and Lipp, 1982). The role of Vesicular Arbacular Mycorrhiza (VAM) is very important for the healthy growth of plant species. Arbacular Mycorrhizas (AM) helps to capture various necessary elements like Nitrogen (N) and Phosphorus (P) by adopting crucial ecological cycles. It has an ability to capture poorly mobile phosphate ions that would be otherwise unavailable to plants via an extensive hyphal network outside the nutrient depletion zone around the root (Sanders and Tinker, 1971). The occurrence of VAM is directly designated to the healthy distribution of Plant species.

3. Materials and Methodology

3.1 Field survey

The study was conducted during the year 2012-2013, in various key areas of plant *Bischofia javanica* in South, East and North districts of Sikkim Himalaya, India. The area includes Rumtek (1400 m), Sichey (1200m), Pakyong (1400m) and Sajong (1200m) in east district, Melli forests (600m) in south district and Passingdang- Dzongu (1200m), Lingthem-Dzongu (1400m) in north district. During the field survey the crucial information on traditional implementation of *Bischofia javanica* were collected from local traditional medicinal experts. A digital camera was used to take snaps of *Bischofia sp.*

3.2 Morophology

The study was carried out with the help of taxonomist as well as by reviewing relevant literatures.

3.3 GNU Octave

The graph plotting requires high level interpreted language software. The GNU
Octave is one of the sophisticated software with an ability to perform graphics line interfaces for data manipulation and visualization. GNU Octave, version 3.6.2, was used for manipulation of research database and for preparing bar graph of the Vesicular Arbuscular Mycorrhiza (VAM) study.

3.4 Literature search
Various research literatures of journals were reviewed from Sikkim Bioinformatics web lab, Sikkim State Council of Science & Technology, Gangtok, including Sikkim Bioinformatics DeLCON consortium free online journals supported by Department of Biotechnology, Government of India.

3.5 Geographical Information System (GIS)
The location maps of studied areas have been prepared by using Arc GIS software, version 10.0, from State Remote Sensing Centre, Sikkim State Council of Science & Technology.

3.6 Questionnaires
The questionnaire format was prepared by referring to the format made by Bioinformatics Centre on Medicinal plants database which is supported of LPAC (Local Project Advisory Committee).

Scientific Name, Local Name, Common Name, Family, Description, Distribution in Sikkim, Distribution in other States, Flowering Period, Fruiting period, Medicinal uses, Parts used and Status.

3.7 VAM (Vesicular Arbuscular Mycorrhiza) study
Roots of Bischofia javanica were collected from Sichey area of East Sikkim and cutted into 1cm pieces. Roots were cleared in 10% (w/v) KOH for 15 min and autoclaved at 14 lb (pound pressure) for 20 min. Drained out the solution and covered the roots with 2%(v/v) HCl (Hydrochloric acid) for 30 seconds. (HCl = 98 ml dilute + 2 ml HCl).

Roots were then removed from HCl and wash with purified water/distilled water and covered the root with alkaline H2O2+ NH4OH (Carbonium hydroxide) for 1 minute. (Alkaline H2O2 was made by adding 3 ml of NH4OH to 30 ml of H2O2 and 567 ml of tap water). Wash the root with distilled water. Treat the bleached roots 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) for 15 minutes at 120° C. (0.05% = 50mg in 100 ml of Distilled water). The root segments were mounted on a slide with 50% (v/v) glycerol for destaining and to be viewed under microscope. Three readings (Reading – I, II, III), were observed both for Vesicular Mycorrhiza and Arbuscular Mycorrhiza.

The density percentage was VAM was obtained as per calibration below;

\[
\text{Percentage (\%)} = \frac{\text{Total no. of Species} \times \text{Total no. of Readings}}{100}
\]

4. Results and Discussion

4.1 GIS (Geographical Information system) mapping
The location map was prepared with the support of Arc GIS software, version 10.0 from State Remote Sensing Centre, Sikkim State Council of Science & Technology, Department of Science & Technology and Climate Change, Sikkim (Figure. 2). GIS has major scientific areas to integrate including ecological research. GIS software can be used to manage and develop digital map related to altitude, vegetation, river route, boundaries etc.

Figure 1: A Bischofia javanica Blume from Sikkim

Figure 2: Location map of studied areas in Sikkim
The study found that in Sikkim Himalaya, the Lepcha tribe used *Bischofia javanica* as traditional medicines in curing various diseases. Under influence of this later it was practiced by all the localities. The medicines used by Primitive Tribe Lepcha are considered to be a most precised and complete form of medicines. It bypasses all sort of the side effect of human health.

**Table 1:** Information detail of questionnaires based on village experts on *Bischofia javanica* Blume

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th><em>Bischofia javanica</em> Blume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Name</td>
<td>Kaijal (Nepali), Sumon kung(Lepcha)</td>
</tr>
<tr>
<td>Family</td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td>Common Name</td>
<td>Bishop Wood</td>
</tr>
<tr>
<td>Description</td>
<td>A tree common in moist shady ravines, river banks and swamps. Distributed to temperate region</td>
</tr>
<tr>
<td>Distribution in Sikkim</td>
<td>Rumtek, Melli forests, Passingdang, Pakyong, Sichey and Sajong</td>
</tr>
<tr>
<td>Distribution in other States</td>
<td>Karnataka, Kerala, Tamil Nadu, Assam, West Bengal, Arunachal Pradesh and Manipur Himalaya, India, East to China, Taiwan.</td>
</tr>
<tr>
<td>Flowering Period</td>
<td>April-June</td>
</tr>
<tr>
<td>Fruiting period</td>
<td>June-October</td>
</tr>
<tr>
<td>Medicinal uses</td>
<td>The leaves are astringent; they are used for sores, toothache and some eye diseases.</td>
</tr>
<tr>
<td>Parts used</td>
<td>Leaves, bark.</td>
</tr>
<tr>
<td>Status</td>
<td>Vulnerable</td>
</tr>
</tbody>
</table>

**Table 2:** VAM occurrence percentage of *Bischofia javanica* Blume

<table>
<thead>
<tr>
<th>VAM</th>
<th>Reading - I</th>
<th>Reading – II</th>
<th>Reading - III</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicular Mycorrhiza</td>
<td>50</td>
<td>25</td>
<td>32</td>
<td>107</td>
<td>3.21</td>
</tr>
<tr>
<td>Arboasclular Mycorrhiza</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>19</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Though, the species is broadly distributed across the state, the key pot areas were selected as per the priority suggested in the literature and by the traditional experts.

*Bischofia javanica* forms a shiny green leaflets. The local children used to eat its fruits. As been found, if necessary, the fruit can be even taken as substitute of meal. It is derived from plants that were first used in traditional systems of medicine (Fabricant and Ramsworth, 2001). The information collected from the traditional experts could pay a good support to the medical science research, in understanding and manufacturing complete drugs and medicines. The *Bischofia javanica* tree were found inhabited in moist shady ravines, river banks and swamps. The information gathered from the local expert based on the prepared questionnaire format is shown in table-1.

**Figure 4:** Bar chart of prevalent VAM in one of the studied area, Sichay, East Sikkim
delicious in taste with full nutritional value. Whenever, diseases like sores, toothache and some eye diseases bursed upon in the villages, an application of Bischofia javanica was found and considered to be the first aid medicines for the remote villagers. Its leaves were used as an astringent for any burn or biting emergencies. The study also found that in Sikkim Himalaya mostly the plant parts being used for medicinal purposes were leaves and bark. There is no such authentic record of uses from root parts, although in some part of the world the roots are also used for medicinal purposes.

4.2 Morphology
The morphology of the species was studied with the help of taxonomist and literature. Morphologically it is a large evergreen tree, bark dark brown hear smooth, Leaves alternate, trifoliate, petiole 6.3-15 cm. Long, stipules 0, leaflets 7.5-12.5 by 3.8-7.5 cm, elliptic or elliptic oblong, acuminate, crenate, glabrous, rather soft, lateral nerves 6-8 pairs; petioles of the lateral leaflets 5-10 mm long of the terminal 2.5-3.8 cm long. Flowers minute, dioecious in axillary or lateral paniculate racemes about as long as the petioles. Male flowers 2.5 mm diameter, rather crowded, pedicels 2.5 mm long; sepals 5, concave, covering the young stamens imbricate; petals 0; disk 0; stamens 5; filaments short, anthers large dehiscing flowers 5mm diameter not crowded; pedicels rather longer than in the male, elongation in fruit; sepals flat ovate, acute, not persistent in fruit, petals 0; disk 0; ovary exerted, glabrous, 3-4 celled; ovules 2 in each cell; styles long; linear, entire. Fruit 7.5-mm diameters, brown or black, globose, smooth, seeds 3-4 smooth, shining.

4.3 Vesicular Arbascular Mycorrhiza (VAM)
The Vesicular Arbascular Mycorrhiza study was conducted in A East Pakoung, India, which was one of the studied area of this research. The microbial laboratory experiment helps to find out the vesicular and arbuscular mycorrhiza (Figure 3). The experiment result shows that percentage of Vesicular Mycorrhiza is high as compared to the Arbascules (Table.2). Vesicles with 3.2% in occurrence and Arbascules (0.57%).

Moreover, the microbial findings of number of respective VAMs through Readings I, II and III methodology revealed that even in all respective readings of VAM, the Vesicles are occurred in number than the Arbascules (Figure 4). It helps to implicate upon importance of presence of exact VAM in Bischofia javanica. This observation reveals that the healthy growth of Bischofia species is much influenced by dense occurrence of Vesicules microbes. More than 80% of the land plant acquires important nutrients from soil with the support of arbuscular mycorrhizas, leading to evolutionary success of this mutualistic symbiosis (Jakobsen., 1995; Smith and Read., 1997).

The study of VAM prevalence characteristics can be a significant approach to assume research on habitats characteristics of microbes, when there is a continuous recedes in the population density of Bischofia javanica in the Himalaya.

Conclusion
Bischofia javanica Blume is a valuable medicinal tree plant of Sikkim Himalaya. Traditionally it is used as an astringent and for the treatment of sores, toothache and some eye disease. Its applications are further broadened with various medicine aspects in other state and region of the world. The healthy growth of species depends upon the microbial mechanisms meet by VAM (Vesicular Arbascular Mycorrhiza) especially the role of Vesicular mycorrhizas. The Bischofia species can be a good natural resource of medicines in Sikkim Himalaya if the species is granted importance and conservation for scientific research.

Acknowledgment
The study acknowledges the support of Bioinformatics Sub DISC, Sikkim State Council of Science & Technology for providing valuable information and the access of free online journals, supported by DeCON, Department of Biotechnology, Government of India. It also acknowledges the Sikkim State Council of Science and Technology for allowing their molecular lab to analyze and develop location map of studied fields by using Geographical Information System (GIS) software.

Author’s Contribution and Competing Interests
The study shall be an important contribution towards the medicinal research sector, which are dedicated to discover organic medicinal species, capable of indicating no side effect to human health. It encourages the society to ponder upon the potential medicinal value and the usability of species Bischofia javanica in
the Himalaya as well as to uphold conservation prospectus of the species in the region. It will promote fundamental research guidelines to initiate strong interest and challenges among the young researcher towards this valuable species.

References


