INTRODUCTION

Plants have played a significant role in maintaining human health and have served as valuable components of neutraceuticals, seasonings, beverages, cosmetics and dyes. The use of plants as source of medicinal agents lies in the roots of antiquity. No one will ever know, what actually led primitive humans to select certain plant materials like roots, leaves, bark, fruits and even plant exudates for the treatment of various ailments and diseases. The history of herbal medicines is as old as human civilization. Many ancient epics, treatise and documents revealed the use of plants long before the beginning of the Christian era. The well-known treatise ‘Charaka Samhita’ and ‘Shushrutha Samhita’ mentioned the medicinal properties of 341 and 395 herbs respectively.

All around the world there is talk about ‘health for all’ but it has been realized that modern pharmaceuticals are remain out of reach of a large proportion of the human population for the near future. This necessitates the use of other sources of human knowledge to provide common health benefits. Thus, herbal medicine has now regarded as important but underutilized tool against disease. The World Health Organization (WHO) recognized this fact in the early 1970s and encouraged governments to effectively utilizing the local knowledge of herbal medicines for disease prevention and health promotion. Presently, the primary health care of 70-80 percent of the world’s population has based on the use of medicinal plants derived from traditional systems of medicine and local health practices. During the past few decades, public interest in traditional, complementary and alternative medicine (TCAM) and use of herbal
medicines has increased dramatically in developed countries. Herbal medicine has based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. The WHO also pointed out that the global sales of herbal products including herbal medicine has already crossed 100 billion in the last five years and has expected to exceed one trillion in the next 20 years at the present growth rate. In India, the herbal drug market is about one billion dollars and the export of plant based crude drugs is around 80 million dollars (Gupta et al., 2010). Therefore, many pharmaceutical companies are showing interest in the production and marketing of herbal medicines. However, in their natural occurrence, plants are found scattered and it is difficult as well as uneconomical to collect and process for the supply from natural resource. It has mentioned that natural habitats for medicinal plants are disappearing fast and together with environmental and geopolitical instabilities. It is increasingly difficult to acquire plant-derived compounds. This has prompted industries, as well as scientists to consider the possibilities of investigation into plant tissue cultures as an alternative supply for the production of plant pharmaceuticals. Advances in Biotechnology particularly methods for culturing plant tissue cultures, should provide new means for the commercial processing of even rare plants and the chemicals they provide. These new technologies will extend and enhance the usefulness of plants as renewable resources of
valuable chemicals. Hence, investigations of a rapid propagation method are very essential for the conservation of the indigenous endangered medicinal plant.

The Western Ghats of Karnataka considered as the storehouse of medicinal plants and harbors nearly 3900 species of flowering plants belongs to 1323 genera and 199 families. Off which, 56 genera 2100 species are found to be endemic (UNESCO World Heritage List, 2010). Due to over exploitation of forest resources, implementation of mega projects and anthropogenic activities many of the endemic plants of this region are at the verge of threatening status. Among the vast assemblage of endemic plants, threatened woody liana legumes of this region such as, *Entada purseath*, *Dalbergia lactea*, *Acacia concinna*, *Acacia wightii*, *Bauhinia varrigata*, *Caesalpinia bonducella* etc. deserve attention by the researchers because of their morphological characteristics and medicinal properties. The present investigation is focusing on a threatened medicinally important legume species *Caesalpinia bonducella*.

1.1. *Caesalpinia bonducella* (L.) Roxb.

1.1.1. Scientific classification

<table>
<thead>
<tr>
<th>Kingdom</th>
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<td>Phylum</td>
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<td>Genus</td>
<td><em>Caesalpinia</em></td>
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<td>Species</td>
<td><em>Caesalpinia bonducella</em> (L.) Roxb.</td>
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1.1.2. Vernacular names

- **English:** Bonduc nut, Fever nut, Nicker nut
- **Sanskrit:** Kuberakshi
- **Hindi:** Katikaranja, Kankarej
- **Tamil:** Kalarciver, Kalarcik Koluntu, Kalarcip paruppu, Kazharchikkaai
- **Telugu:** Mulluthige, Gaccakayai
- **Malayalam:** Bankaretti, Kaka-moullou, Kazhanji, Kalanci, Kajanchikkur
- **Kannada:** Gajuga.

1.1.3. Botanical description

*Caesalpinia bonducella* is a spinucent woody liana very sparsely distributed in the Western Ghats of Karnataka (Fig. 1A). The leaves are bipinnately compound measuring 3-60 cm long with short prickly petioles. Pinnae are 68 pairs with a pair of hooked stipulary spines at the base. Leaflets are 6-9 pairs, 2-3.8 cm long and 1.2-3.2 cm wide, elliptic, oblong, strongly mucornate and glabrous above.

Inflorescence a supra-axillary or terminal raceme or panicle 30–60 cm long, densely flowered (Fig. 1B). Flowers are bisexual or functionally unisexual, zygomorphic, 5-merous. Sepals free, unequal, c. 5 mm×2.5 mm, the lowest one hood-shaped, pubiscent, oblong and ovate. Petals free, unequal, 6–7 mm × 2–3 mm, clawed, yellow, the upper one different in shape and size, petals oblancolate, filaments declinate and flattened at the
Fig. 1. *Caesalpinia bonducella* growing in the Western Ghats of Shimoga, Karnataka

A. Habitat of *C. bonducella*; B. Inflorescence of *C. bonducella*; C. Pods of *C. bonducella*; D. seeds of *C. bonducella*
base. Stamens 10, free, c. 5 mm long, filaments hairy towards base. Ovary superior, monocarpellary, unilocular with ovules on marginal placenta, style short. Fruit an oblong, inflated pod 5–8 cm × 3–4.5 cm, dehiscent, covered with stiff, long hairy prickles, 1–2 seeded (Fig. 1C). Seeds are ovoid, 1.5 – 2 cm in diameter, smooth, hard, pale grey. Seedling with epigeal germination; cotyledons rounded, thick, oblong dark grayish in color (Fig. 1D).

*Caesalpinia bonducella* can be found flowering and fruiting throughout the year. Seeds of *Caesalpinia bonducella* float and retain their viability in water for extended periods. This species is found to be distributed in tropical America, tropical Africa and tropical Asian countries. *Caesalpinia crista* L. mentioned in literature on Africa is a misapplied name and can refer to either the indigenous *Caesalpinia bonducella* or the introduced *Caesalpinia decapetala* (Roth) Alston. In tropical Africa *Caesalpinia crista* has only been recorded in the Seychelles and Mauritius and is otherwise found in the Americas, Asia, Australia and the Pacific Ocean islands.

### 1.2. Need for investigation

#### 1.2.1. Traditional medicinal claim

The seeds of *C. bonducella* plant are being used in Ayurvedic system of medicine for the treatment of contagious diseases, inflammation, leprosy, antiperiodic, febrifuge, anthelmenthic, urinary disorders, leucorrhoea, tumors, piles and to heal wounds. The seed is claimed to be styptic, purgative and anthelmintic and cures inflammations, useful in colic, malaria, hydrocele, skin diseases and leprosy. In Madras (Chennai) an ointment is made from the powdered seeds with castor oil and applied externally in hydrocele and
orchitis (Handa et al., 1996; The Wealth of India, 1992; Kapoor, Ayurvedic Medicinal Plants; Elizabeth 83-86; Kirtikar et al., 1993). The seeds are considered tonic, febrifuge, anthelmintic, antiblennorrhagic, and specific in the treatment of hydrocele. The oil from the seeds has been used for convulsions and paralysis.

The oil from the seeds is used in convulsions and paralysis. Kernel powder with sugar and goat milk gives good results in liver disorder (Khan et al., 1982). Decoction of roasted kernels was used to treat asthma. Traditionally, leaf juice is used to cure elephantiasis, liver disorders and smallpox. Flowers are used to control Vata and Kaffa (Moon et al., 2010). Bark of root possesses number of properties like febrifuge, intestinal worms, amenorrhoea, cough etc., Flowers are used in treating ascites and fruits in treating urinary disorder, leucorrhoea, piles and wounds. Leaves and twigs are used for the treatment of tumors, inflammation and liver disorder. In Ayurveda, literatures of medicinal property of C. bonducella was described as Rasa: Tikta (bitter), kashaya (astringent); Guna: Laghu (light), ruksha (dry), tikshna (sharp); Veerya: Ushna (hot), Vipaka: Katu (pungent); Dosha: Pacifies tridosha (Williamson et al., 2002, Nazeerullah et al., 2012). The seeds, leaves and roots used in the treatment of tachycardia, bradycardia, tuberculosis, tympanitis, pain in the abdomen, fever, cold and cough and liver fluke in ruminants reviewed by Nazeerullah et al., (2012).

In Africa its leaves, bark and roots are used to cure fever, headache and chest pain and as an anthelmintic. In West Africa, it has used as a rubefacient and as a tonic in the treatment of jaundice, diarrhoea and skin eruptions. At the Kenyan coast, the seed and decoctions of the leaves and roots are taken to treat asthma and complications during menstruation, to avoid miscarriage, and as eye-drops to treat internal blood clots in the
eye. In Tanzania, the powdered kernel of the seeds is taken with water to treat diabetes mellitus. In Somalia, the oil from the seeds is used to treat rheumatism. A bitter extract from the seeds is known as ‘poor man’s quinine’ and is used against malaria e.g. in India, but its use in Africa as a malaria cure has not been documented.

In Guinea, the pounded seeds are considered as vesicant. The powdered seeds were mixed with equal part of pepper powder and administered to malaria patients and were found to posses feeble antiperiodic properties. In malignant malaria, they did not do any good. The seeds are ground in water and given internally in snake-bite. In West Indies, the roasted seeds are used as anti diabetic (Komal Moon et al., 2010). The kernel of the seed is very useful and valuable in all ordinary cases of simple, continued and intermittent fevers. The kernel powder was mixed with equal parts of black pepper is taken thrice a day in a dose of 15-30 grains by adults and 3-4 grains by children.

In Jamaica, it is used as a rubifacient and as a local application for sores. Flowers are bitter tastes and induces warming effect on the body. It improves the balance of vata and kapha and the ash is useful in ascites and fruits in treating urinary disorder, leucorrhoea, piles and wounds. Leaves and twigs are traditionally used for the treatment of tumors, inflammation and liver disorder. They have also been applied for treatment of toothache. Leaves and juices have been used traditionally for elephantiasis and smallpox. The leaves and the juice or paste is used for treating smallpox, elephantiasis, liver diseases and for eliminating bad odour in perspiration. It is also used for reducing toothache C. bonducella has febrifuge, antiperiodic, anthelmintic and tonic properties. Pods are roasted and the powder is used as a substitute for quinine. The root bark has been used for treating intestinal worms, fever, tumors, cough, amenorrhea, and to remove
placenta after childbirth. The leaves and seeds after roasting in castor oil can be applied
to reduce piles, inflammatory swellings, orchitis and hydrocele. Boiled leaves can be
used for gargling to relieve sore throat. The fruit is also used for eliminating piles,
wounds, leucorrhoea and urinary disorders.

In tropical Asia and the Pacific Ocean islands, *C. bonducella* seeds are widely
used as beads, as weights and as counters in board games (Francis, 2003; Pakia & Cooke,
2003). It was made official in the Indian Pharmaceutical Codex 16 that at the dose of
15-18 mg/kg/wt. seed powder is said to produce perspiration, leading to the reduction of
fever. Kernel powder with sugar and goat milk gives good result results in liver disorder
(Khan *et al.*, 1982). Decoction of roasted kernel was used in asthma. Children unable to
digest mother’s milk was given the extract of the kernel or its powder along with ginger,
salt and honey to get good stomachic effect. Paste prepared from kernel gives relief from
boils and other such swellings. A cake made of 30 grains of powdered kernels, fried in
ghee taken twice a day is a valuable remedy in cases of acute orchitis, ovaritis and
scrofula. Root (Moon *et al.*, 2010) in La Reunion and Madagascar, the roots are
considered febrifuge and anthelmintic, they are much used as an astringent in leucorrhoea
and blennorrhagia.

Reports are very scarce on the medicinal uses of the stem bark and leaves of this
species. The traditional practitioners residing in the vicinity of the Western Ghats of
Karnataka are using the leaves to cure jaundice and liver disorders and stem bark has
been used to cure diabetic wounds and wring worm and dermatitis.
1.2.2. Phytochemicals and pharmacological evaluation reports

Many investigators rigorously screened the phytoconstituents of the seeds and evaluated the pharmacological properties of the seed extracts of *C. bonducella*. The detail reports are explained in the Chapter -2, Review of literature. The leaves and stem bark of this species are yet to be explored for novel phytoconstituents. The therapeutic efficacy of the different solvent extracts and the phytoconstituents of the leaves and the stem bark and their in *vitro* derived calli have not been investigated in detail.

1.2.3. Mass multiplication of calli and plantlets

*C. bonducella* is a woody legume propagated through the hard nut like seeds and are remained dormant for many years in the soil. Because of over exploitation medicinally important parts and destruction of habitat, this species reached the status of threatening condition. Application of tissue culture technology is one the important tool in the *ex situ* conservation of threatened medicinal plants. The earlier investigator Kannan *et al.*, (2006) has conducted preliminary studies in establishing *in vitro* regeneration protocol using mature stem explants of *C. bonducella* through direct organogenesis. Cheruvathur *et al.*, (2010) has developed an efficient plant regeneration method by culturing immature epicotyl explants of *C. bonducella*. The exploration of morphogenic potentialities of the different explants for *in vitro* regenerative efficacy has not investigated in detail. Further, the biosynthetic potentialities of the *in vitro* derived calli for the production of therapeutically active compounds are yet to be investigated.

In view of the above, the present investigation was designed on micropropagation, phytochemical screening and evaluation of pharmacological properties of *Caesalpinia bonducella*. 

1.3. Objectives

1. To study the regenerative potentialities of the seedling and mature plant of *Caesalpinia bonducella.*

2. To study the effect of growth regulators on mass multiplication of leaf and stem calli.

3. To screen the phytocompounds from the leaves, stem bark and their *in vitro* derived calli extracts.

4. To evaluate the pharmacological property of the leaf, stem bark extracts and the isolated compounds for the following preclinical models using experimental rats.
   a. *In vitro* antioxidant assay.
   b. Hepatoprotective and *in vivo* antioxidant activity.
   c. Anticancerous activity on human cancerous cell lines.
   d. Diabetic wound healing activity.
   e. Antimicrobial activity.