Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Callus Induction and Shoot Regeneration from the Immature Flower Bud of *Caesalpinia bonducella* and its Antileptospiral Potential by *in vitro* and *in silico* Analysis

S. R. Santosh Kumar, Chandrashekar Srinivasa[1], Chandan Shivamallu[2], Kollur Shiva Prasad[3], Sushma Pradeep[2], Asad Syed[4], Ali Bhakali[4], Amar Shankar[5], Sharanagouda S Patil[6], Ashwini P[7], Chandan D[2], Triveni R[8], Govindappa Melappa[9], V. Krishna[10]

Departments of Studies in Food Technology and [1]Studies in Biotechnology, Davangere University, Shivagangotri, Davangere, [2]Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, [3]Department of Sciences, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru, Karnataka, India, [4]Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia, [5]Department of Food Technology, School of Engineering and Technology, JAIN (Deemed-to-be University), [6]ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, [7]Department of Microbiology, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, [8]Sri H.N. Ananthkumar, P.U.College, Bidadi, Bengaluru, [9]Department of Studies in Botany, Davangere University, Shivagangotri, Davangere, [10]Department of Biotechnology, Kuvempu University, Shivamogga, Karnataka, India

Submitted: 10-Aug-2020 Revised: 04-Sep-2020 Accepted: 24-Feb-2021 Published: 10-Jun-2021

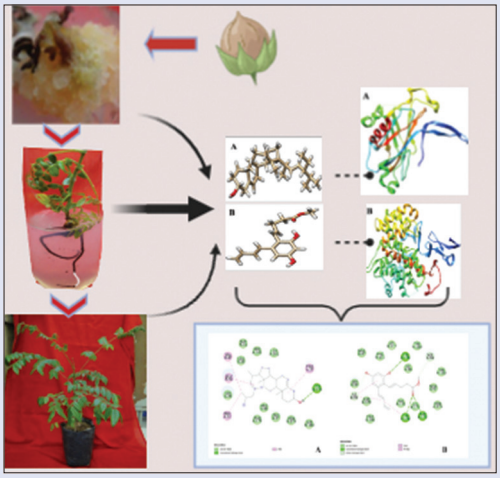
ABSTRACT

Background: An economical plant regeneration was established for *Caesalpinia bonducella* by culturing immature flower buds and phytoconstituents such as β-Sitosterol (LC3) and methyl (4*E*)-5-(2-[(1*E*)-buta-1,3-dien-1-yl]-4,6-dihydroxyphenyl) pent-4-enoate (SC2) were isolated from its extracts and the isolated phytoconstituents were tested against *Leptospira interrogans.* **Objectives:** The aim of the study is to establish the *C. bonducella* through tissue culture technique and to investigate the antileptospiral activity through computational and *in vitro* screening. **Materials and Methods:** Morphogenic calli were initiated from 96% of immature flower buds on MS medium supplemented with 2, 4 D (2, 4-Dichlorophenoxyacetic acid) 2.0 mg/l and 6-Benzylaminopurine (BAP) 0.5 mg/l. The calli formed were excised and subcultured on MS medium. Extreme percentage organogenesis (84%) and average shoots per culture were determined on MS medium fortified with 3.0 mg/l BAP and 0.5 mg/l indole-3-butyric acid (IBA). The addition of IBA in ½ MS medium favored the development of recovered shoots. Out of 30 shoots transferred to soil, 27 survived once acclimated. The isolated compounds were selected for *in vitro* and *in silico* screening. The primary pharmacological assay for leptospirosis was carried out by test tube dilution and microdilution technique and the computational screening was done using molecular docking. **Results:** The phytoconstituents obtained from the medicinal plant showed promising results in both *in vitro* and *in silico* antileptospiral activity. **Conclusion:** The LC3 and SC2 compounds isolated from *C. bonducella* were evaluated on Gram-negative bacteria *L. interrogans*. The assay and molecular docking studies revealed the efficacy of phytocomponents of *C. bonducella* as traditional medicine which has an ability to cure bacterial diseases.

Key words: Antileptospiral activity, *Caesalpinia bonducella,* callus culture, *Leptospira interrogans*, molecular docking

SUMMARY

- Three novel biologically active compounds were isolated from *Caesalpinia bonducella*
- The *in silico* antibacterial activity of *C. bonducella* isolated compounds LC3 and SC2 were evaluated on Gram-negative bacteria *Leptospira interrogans* through molecular docking studies
- The *in vitro* and *in silico* analysis suggested that the phytocomponents of *C. bonducella* can be effectively used as traditional medicine which has the ability to cure bacterial diseases.



Abbreviations used: PDB: Protein data bank; BAP: 6-Benzylaminopurine; IBA: Indole-3-butyric acid; TDT: Test tube dilution; MDT: Microdilution technique.

Correspondence:

Prof. V. Krishna,
Department of Biotechnology, Kuvempu University, Shankarghatta, Shivamogga - 577 451, Karnataka, India.
E-mail: chandans@jssuni.edu.in

DOI: 10.4103/pm.pm_331_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The elements of the plant *Caesalpinia bonducella* area unit being employed in the Ayurvedic system of drugs for the treatment of contagious diseases, inflammation, leprosy, antiperiodic, febrifuge, anthelminthic, urinary disorders, leukorrhea, tumors and piles, and to heal wounds.[1] The seed is claimed to be styptic, purgative, and anthelmintic and cures

Cite this article as: Kumar SR, Srinivasa C, Shivamallu C, Prasad KS, Pradeep S, Syed A, *et al*. Callus induction and shoot regeneration from the immature flower bud of *Caesalpinia bonducella* and its antileptospiral potential by *in vitro* and *in silico* analysis. Phcog Mag 2021;17:S38-44.

inflammations, helpful in hurting malaria, hydrocele, skin disease, and infectious disease. In Chennai, the ointment is formed from the powdery seeds with physic and applied outwardly in hydrocele and redness and pain in testis.[2] The seeds are considered tonic, febrifuge, anthelmintic, antiblennorrhagic, and specific in the treatment of hydrocele.[3] The oil from the seeds has been using for convulsions and paralysis. In Africa, its leaves, bark, and roots are accustom used to cure fever, headache, and chest pain and as an anthelminthic. In West Africa, it is used as a rubefacient and as a tonic in the treatment of jaundice, diarrhea, and skin eruptions.[4] At the Kenyan coast, the seed and decoctions of the leaves and roots are accustom used to treat asthma attack and complications throughout the flow, to avoid miscarriage, and as eyedrops to treat internal blood clots within the eye. Reports say terribly scarce on the healthful uses of the stem bark and leaves of this species. The standard practitioners residing within the locality of the Western Ghats of Karnataka are consuming the leaves to cure jaundice and liver disorders and stem bark has been used to cure diabetic wounds and wring worm and eczema. Inflorescence a supra-axillary or terminal flower cluster or raceme 30–60 cm long, densely floral, flowers are bisexual or functionally sexual, bilaterally symmetrical, 5-merous. Sepals free, unequal, 5 mm × 2.5 mm, the bottom one hood-shaped, pubescent, rectangular, and ovate. Petals free, unequal, 6–7 mm × 2–3 mm, clawed, yellow, the higher one totally different in form and size, petals simple, filaments declinate at the bottom.[1] Stamens 10, free 5 mm long, filaments furry toward the bottom. Ovary superior, monocarpellary, unilocular with ovules on the marginal placenta, vogue short.

C. bonducella will be found flowering and mature throughout the year. Seeds of *C. 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. *C. bonducella* could be a woody legume propagated through the hardest-like seeds and remained dormant for several years within the soil. Thanks to the overutilization medicinally vital elements and destruction of the environment, this species reached the standing of the threatening condition. The application of tissue culture technology is one vital tool within the *ex situ* conservation of vulnerable medicative plants. The sooner investigators Klein *et al.* have conducted preliminary studies in establishing *in vitro* regeneration protocol using mature stem explants of *C. bonducella* through direct organogenesis.[5] Cheruvathur *et al.* have developed an economical plant regeneration[5,6] technique by culturing immature epicotyl explants of *C. bonducella*. The exploration of morphogenic potentialities of the various explants for *in vitro* regenerative effectuality has not investigated intimately.[1] Further, the synthesis potentialities of the *in vitro* derived calli for the assembly of therapeutically active compounds however to be investigated. In sight of the on top of, the current investigation was designed on regenerative potentialities of *C. bonducella* using flower bud as explant.

C. bonducella is a woody legume propagated through the hard nut-like seeds and is remained dormant for many years in the soil. Because of overexploitation medicinally important parts and destruction of habitat, this species reached the status of the threatening condition. The application of tissue culture technology is one of the important tools in the *ex situ* conservation of threatened medicinal plants. Work has been carried out on floral morphogenesis using (1) floral apices and (2) vegetative explants.

To exploit the advantages of these two kinds of explants, they were grown under different controlled physiological conditions using various types of cultural media. Current investigations were done to study their growth capacity which are arbitrarily classified as culturing of induced explants to study persistence of stimulus, culturing of vegetative parts in a view of induction of flowering and further initiation studies and culturing of flower buds.

The flower morphological studies can be done through a technique used to culture young excised floral primordial that has become a potentially useful tool. It helps in assessing the influence of buds on nutrients and growth factors in the absence of intervening vegetative tissues. Second, the behavior of the floral apex can be autonomously tested to check if the sequence of organ initiation and subsequent growth is regulated inside the isolated immature bud. Last, it allows a variety of surgical manipulations and ensures easy handling of the excised buds in culture.

Before conducting the *in vitro* studies, the potentiality of the drug was confirmed by computational screening technique. The isolated phytoconstituents from *C. bonducella* were screened for *in vitro* antileptospiral activity against Gram-negative bacteria, namely *Leptospira interrogans.* Leptospirosis is bacterial infection caused by the members of leptospira bacteria. The bacteria are transmitted through the blood or urine of infected animals. Citrate synthase is the major enzyme required for the synthesis of glutamate from glycolytic intermediates in the Krebs cycle as this cycle is involved in the production of ATP, it is one of the major contributor in enhancing the growth of leptospira species. LipL32 is the most prominent outer membrane protein of leptospiral protein profile and is involved in the pathogenesis, diagnosis, and prevention of leptospirosis.[7] Both citrate synthase and LipL32 proteins/enzymes were considered for the further *in silico* docking studies to check the inhibition activity of the *C. bonducella* isolated phytoconstituents.

MATERIALS AND METHODS

Collection of plants

The tender, explants of *C. bonducella* were collected from elite plant maintained in the University Garden of Shimogha University, Karnataka, India. The plant material that is to be cultured was totally washed in running water, followed by 5% liquid detergent "Tween-20" for a couple of minutes to get rid of all detritus. The desired segmental elements were surface sterilized with 0.1% (W/V) aqueous mercuric chloride ($HgCl_2$) for 8–10 min, followed by 5–6 rinses with sterilized water and were taken into antecedently ultraviolet radiant sterilized laminar airflow chamber. With the assistance of a sterilized blade, totally different parts of the explants were withdrawn cross-segments of needed length and were fastidiously inoculated onto the MS organogenic and callogenic media. The impact auxins and cytokinins on organogenic and callogenic potentialities of every of the juvenile and mature plant explants were tested individually. Immature flower buds (3–5 mm) of *C. bonducella* were collected from the mother elite plant grown within the forest vary of Bhadra Wildlife Sanctuary within the month of December. The flower buds are aseptically cultured on callogenic media. Floral callus was initiated on the media at the expansion regulator levels of 0.5–3 mg/l 2, 4-D, and 0.1–1.0 mg/l 6-Benzylaminopurine (BAP). The primary calli subcultured on to the shooting media that containing MS basal salts supplemented with a range of 2–5 mg/l BAP and 0.3–0.9 mg/l indole-3-butyric acid (IBA). After 4 weeks of shoot initiation, the shoot lets grew higher than 3–4 cm length were transferred to rooting maturation media increased with a range of 0.1–1.0 mg/l of IBA. The regenerants were hardened at greenhouse condition and transferred to the pots containing sterilized soil.

The stem bark and leaves of *C. bonducella* were obtained from forest ranges of the Western Ghats (1 km from Kuvempu University) of Bhadra Wildlife Sanctuary, Karnataka. The plant was authenticated by Prof V. Krishna, author of Flora of Davanagere District, Karnataka, and Prof. Y. L Krishnamurthy, Department of Applied Botany, Kuvempu

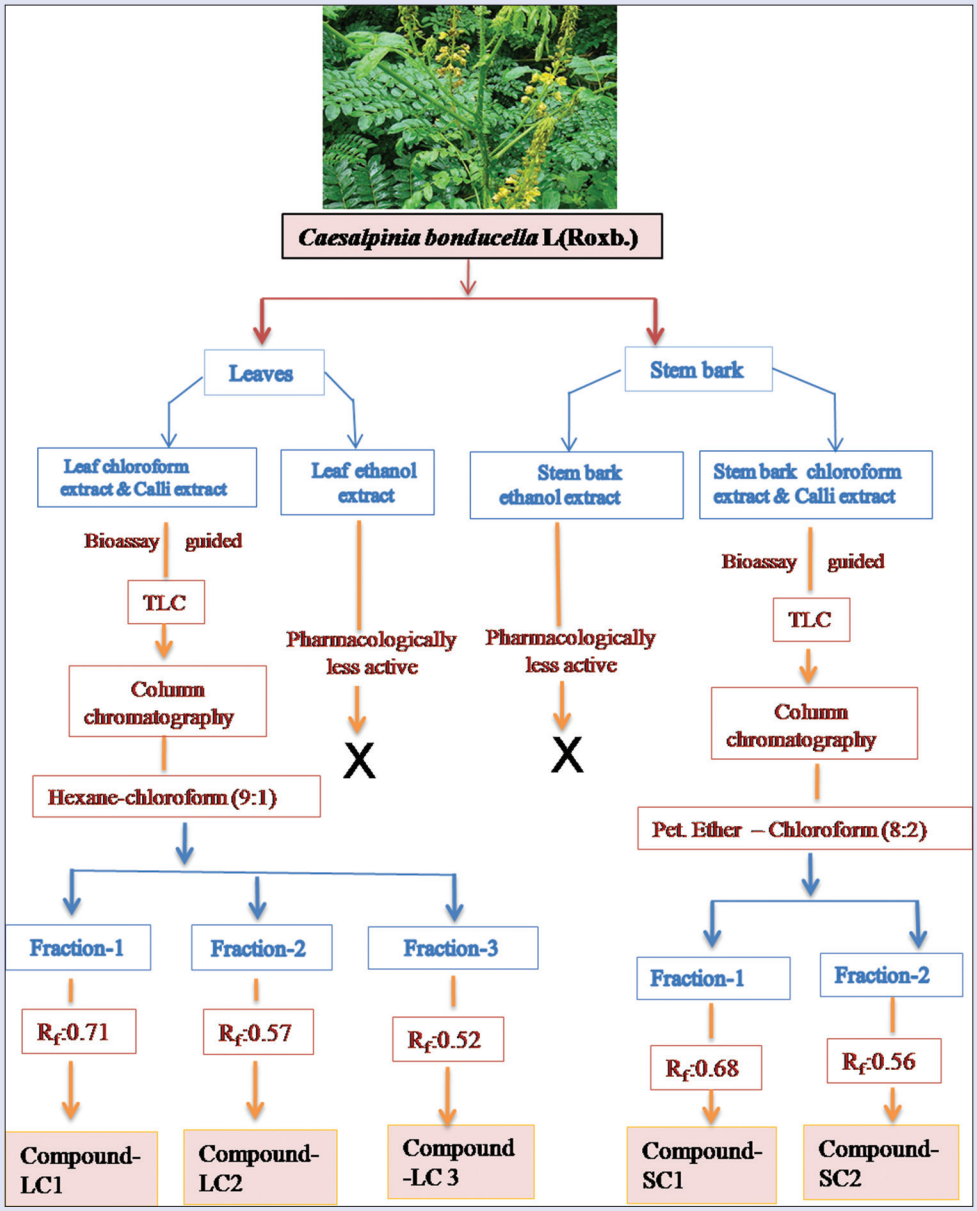
University, Shankaraghatta, Shivamogga, District, Karnataka, comparing with the herbarium voucher specimen (KUAB.301) deposited at Kuvempu University herbaria. The leaves and stem bark materials were shade dried, powdered mechanically (sieve No. 10/44), stored in airtight containers, and subjected to phytochemical investigations. The oven-dried leaf calli and stem calli of *C. bonducella* induced on optimal concentrations of growth regulators were also used for secondary metabolite extraction.

The shade-dried, powdered materials of the plants were subjected to successive solvent extraction as described below. The powdered materials of *C. bonducella* leaves, stem bark shade dried were taken separately in 1 l capacity thimble of soxhlet apparatus and refluxed successively with the solvents petroleum ether (40–60, E-Mark Mumbai, India), Chloroform (E-Mark Mumbai, India), and ethanol (E-Mark Mumbai, India) for 48 h in 16 batches of 600 g each. Each time, the solvent from the marc was removed completely before extracting with the next solvent. With the help of rotary flash evaporator, all the extracts were concentrated and filtered in vacuum. The leftover solvent was completely removed and dried in the desiccators. The crude extracts obtained were labeled, weighed, and the percentage of yield was recorded [Scheme 1].

In silico molecular docking studies

Protein preparation

The phytoconstituents β-Sitosterol (LC3) and methyl (4*E*)-5-{2-[(1*E*)-buta-1,3-dien-1-yl]-4,6-dihydroxyphenyl} pent-4-enoate (SC2) were docked against the *L. interrogans* species to know their antibacterial activity. The protein structure files (.pdb) were downloaded from Protein Data Bank (PDB) based on their resolution values that must be below 2Å. In this study, *leptospiral* proteins such as citrate synthase (PDB ID: 2H12) and lipl32 (PDB ID: 2ZZ8) were considered as the macromolecules while doing the docking studies[8] [Figure 1a and b]. The binding site residues for the selected proteins were obtained from GalaxyWeb binding site prediction tool.[9]



Scheme 1: Detail procedure for the isolation of those three phytochemicals

Ligand optimization

The two phytoconstituents β-Sitosterol and methyl pent-4-enoate isolated from *C. bonducella* were sketched and geometrically cleaned the structure using ChemSketch software.[10] To precede with the molecular docking studies, the 3D structure of ligand is required; hence, the 2D sketched structure was converted to a 3D structure file (.pdb) using OpenBabel software by generating the 3D coordinates [Figure 2a and b]. The ligands and the macromolecules are now fit for the further molecular docking studies.[11]

PyRx software was used to do the molecular docking studies between the ligands and the macromolecule. The respective phytoconstituent and protein were loaded into the PyRx and made them as ligand and protein to obtain the pdbqt files. The binding site pocket amino acid residues were selected in the protein to generate a grid box around them. The Lamarckian algorithm was selected to generate the docked poses of the ligand.[12] After the completion of the docking process, eight docked possess of the ligand with the binding affinity and rmsd values against the protein were obtained as the results. The results were analyzed using Discoverystudio visualization software.[13]

Antileptospiral activity of LC3 and SC2 bioactive compounds

Antileptospiral activity of bioactive compounds was analyzed according to the protocol, followed by Govindaraju *et al.* 2017 using both tube and microdilution.[14] Five *Leptospira* strains procured from the ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI, Bengaluru) were used as a reference standard. In the microdilution technique, viable cells of *Leptospira* were taken in all the wells of the microtiter plate (96 well plate) in such a way that each strain occupied 10 wells of the 96 wells, to this varied concentration of bioactive compounds were added in such a way that each strain received 25, 50, 75, 100, 125, and 150 µg concentrations, respectively (5 µl volume). Benzylpenicillin was commonly used as antileptospiral drug standard and served as positive control. Samples were mixed thoroughly to obtain appropriate activity and were wrapped with fresh silver foil and were incubated for 30 min at room temperature in the dark condition. The cultures were spread on cavity slides after incubation and observed for inhibition of leptospiral activity under dark-field microscope. In tube dilution technique, varied concentrations of bioactive compounds were added to leptospiral media (EMJH media) containing calcium chloride (1%) and rabbit serum (15%) (Sigma, USA) and enriched with L-asparagine (3%), pyruvate sodium (1%), magnesium chloride (1%), and 0.2% agar with the addition of 5-fluoruracil (300 mg/L) named as the selective medium. The sterilized media were checked for contamination by keeping the media at room temperature for 48 h before the inoculation of *Leptospira* strains. The *Leptospira* strains were inoculated to the sterile media and the tubes were incubated for 7 days at room temperature and the tube lacking bioactive compounds was considered as blank and the tube with benzylpenicillin was taken as positive control. After 7 days of incubation, percentage inhibition of the activity of *Leptospira* was analyzed by observing the samples under dark-field microscope. Both microdilution technique and tube dilution technique were conducted in triplicates.

RESULTS AND DISCUSSION

Floral callus was initiated on the media supplemented with a variety of 0.5–3 mg/l, 2,4-D, and 0.1–1 mg/l breadstuff [Figure 3a]. Callogenesis was initiated from the neural structure region of the flower, bit by bit it proceeded toward the non-accessory and accent floral components of the flower [Figure 3b]. Best callogenesis was detected from the bud explants at the concentration of 0.5 mg/l 2, 4-D [Figure 3c and Table 1]. The first floral callus fleshy creamy white and nodular, whereas the shoot differentiating callus was pale achromatic interspersed with dark-green loci [Figure 3d]. The first calli subcultured on to the shooting media that contain MS basal salts supplemented with a variety of 2–5 mg/l BAP and 0.3–0.9 mg/l IBA for shoot induction [Figure 3e] and high-frequency shoot induction was detected at 3 mg/l BAP and 0.5 mg/l IBA [Figure 3f and Table 2]. When 4 weeks of shoot initiation, the shootlets grew on top of 3–4 cm. The length was transferred to growing media increased with completely different concentrations of IBA (0.1–1.0 mg/l) achieved at the concentration of 0.6 mg/l IBA [Figure 4a]. The regenerants were hardened primarily within the synthetic resin luggage at inexperienced house condition and transferred to the pots containing sterilized soil. The regenerants are with success hardened in outside the laboratory [Figure 4b].

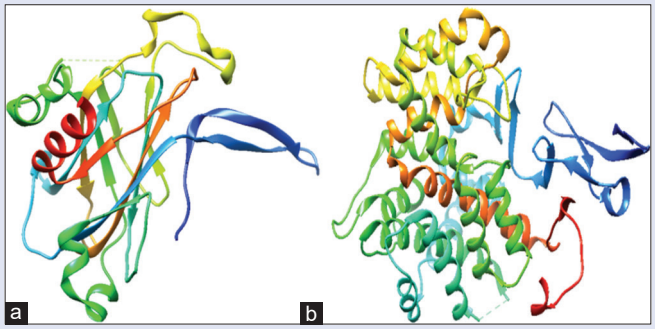


Figure 1: The 3D ribbon preset model of the selected *Leptospira interrogans* proteins (a) 2ZZ8 and (b) 2H12

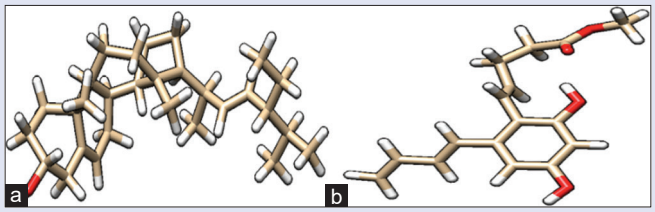


Figure 2: (a) the 3D structure of LC3 (β-Sitosterol); and (b) the 3d structure of SC2 (Methyl (4E)-5-{2-[(1E)-buta-1,3-dien-1-yl]-4,6-dihydroxyphenyl} pent-4-enoate)

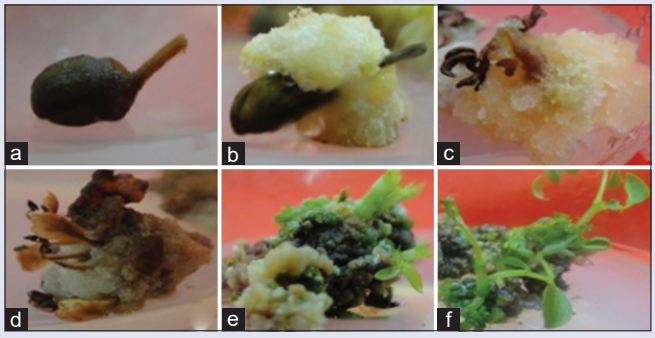


Figure 3: Indirect organogenesis through flower bud explant: (a) Unopened flower bud; (b) Callus formation on Flower bud; (c and d) Stages of callus (e) Initiation of greenish nodal like shoots bud organization; (f) Sprouting of multiple Shoots differentiation

Table 1: Effect of 2, 4-D, and 6-benzylaminopurine on the frequency of callus formation from the flower bud of *Caesalpinia bonducella*

Growth regulators (mg/L)		Frequency of callus formation (%)
2,4-D	BAP	
0.5	0.1	10.0
0.5	0.3	33.3
0.5	0.5	26.6
0.5	0.8	36.6
0.5	1.0	23.3
1	0.1	26.6
1	0.3	20.0
1	0.5	36.6
1	0.8	46.6
1	1.0	23.3
2	0.1	56.6
2	0.3	63.3
2	0.5	80.0
2	0.8	50.0
2	1.0	40.0
3	0.1	70.0
3	0.3	33.3
3	0.5	20.0
3	0.8	16.6
3	1.0	10.0

The value of each combination consisted of the percentage of callus induction from the flower bud of 3×10 replicates. BAP: 6-Benzylaminopurine

Table 2: Effect of 6-benzylaminopurine and indole-3-butyric acid on shoot developed from the flower bud callus of *Caesalpinia bonducella*

Plant growth regulators (mg/L)		Number of shoot buds/ callus (mean±SD)
BAP	IBA	
2	0.3	0.30±0.48
2	0.5	0.50±0.53
2	0.7	0.80±0.42
2	0.9	1.20±0.63
3	0.3	2.00±0.82
3	0.5	3.80±0.79
3	0.7	3.30±0.67
3	0.9	1.20±0.63
4	0.3	2.20±0.63
4	0.5	1.50±0.71
4	0.7	1.20±0.79
4	0.9	0.90±0.74
5	0.3	1.00±0.47
5	0.5	0.80±0.63
5	0.7	0.60±0.52
5	0.9	0.40±0.52

The value of each combination consisted of mean±SD of 10 replicates. SD: Standard deviation; BAP: 6-Benzylaminopurine; IBA: Indole-3-butyric acid

In silico molecular docking studies

The two bioactive compounds were docked against the *L. interrogans* to check their antibacterial activity. The SC2 ligand has shown a remarkable interaction of three and two hydrogen bonds with the selected proteins 2H12 and 2ZZ8, respectively, and has obtained the highest binding energy of −7.7 and −8.0 [Figure 5a and b]. But when compared to SC2, the LC3 exhibited binding affinity of −7.1 by forming only one hydrogen bond with 2H12, whereas with 2ZZ8, there was no formation of hydrogen bond except for hydrophobic interactions with the binding affinity of −7.3 [Figure 6a and b].

Antileptospiral activity

The above results were enough to convince us to check the activity of bioactive compounds against unusual and unique organism such as *Leptospira*, which possesses a unique metabolism. In both the microdilution and tube dilution technique, benzylpenicillin was used as positive control and bioactive compounds as test samples, it was quite fascinating to note that both the bioactive compounds showed promising results and completely inhibited leptospiral growth at concentrations as less as 75 µg which is profoundly significant. It is also quite interesting to note that the bioactive compounds were found to possess higher antileptospiral activity equal to the standard drug [Tables 2a and b].

DISCUSSION

Medicinal plants measure a valuable supply of a massive array of chemical compounds. They synthesize and accumulate extractable organic substances for various drug preparations.[15] In spite of tremendous advances made in modern medicine, there are still a large number of ailments for which suitable drugs are yet to be investigated. The information of the biological activities and therefore the chemical constituents of plants square measure fascinating, not just for the invention of recent therapeutic agents however additionally revealing the worth of new sources of economically useful materials.[16] Thus, a careful and extensive study on medicinal plants is necessary to identify newer plant products to evaluate the efficacy of the compound to combat the disease. In recent years, there has been an awakening all over the world for the use of organic medicaments in place of synthetic and antibiotics and therefore has been consistent growth in demand of many of these plant-based drugs and several plant products from diverse species.[17] This has given rise to large-scale collection of several medicinal plants and many of them are on the verge of extinction. Deforestation and changing environmental conditions have been causing a threat to many species thereby some species have already become extinct and some others are threatened to extinction.[18-20] There has been a growing awareness of the imminent danger to plant life and naturalists are evincing interest in biodiversity and its conservation through *in situ* and *ex situ* method.

Table 2a: Effects of different concentrations of *β*-sitosterol against various species of *Leptospira* through tube dilution technique and microdilution technique

Species name	Technique	Inhibition rate percentage in different concentration of LC3 compound						Standard benzylpenicillin 30
		25	50	75	100	125	150	
Leptospira pomona	MDT	90	95	100	100	100	100	100
	TDT	85	90	100	100	100	100	90
Leptospira javanica	MDT	75	90	100	100	100	100	90
	TDT	75	90	100	100	100	100	90
Leptospira pyrogens	MDT	80	85	100	100	100	100	90
	TDT	80	85	100	100	100	100	90
Leptospira australis	MDT	70	90	100	100	100	100	95
	TDT	70	85	100	100	100	100	100
Leptospira hardjo	MDT	90	95	100	100	100	100	95
	TDT	90	95	100	100	100	100	100

TDT: Tube dilution technique; MDT: Microdilution technique; LC3: *β*-Sitosterol

Table 2b: Effects of different concentrations of pent-4-enoate against various species of *Leptospira* through tube dilution technique and microdilution technique

Species name	Technique	Inhibition rate percentage in various concentration of SC2 compound						Standard benzylpenicillin 30
		25	50	75	100	125	150	
Leptospira pomona	MDT	95	95	100	100	100	100	100
	TDT	90	80	100	100	100	100	90
Leptospira javanica	MDT	90	90	100	100	100	100	90
	TDT	90	83	100	100	100	100	90
Leptospira pyrogens	MDT	85	95	100	100	100	100	90
	TDT	85	85	100	100	100	100	90
Leptospira australis	MDT	90	80	100	100	100	100	95
	TDT	85	95	100	100	100	100	100
Leptospira hardjo	MDT	95	95	100	100	100	100	95
	TDT	95	85	100	100	100	100	100

TDT: Tube dilution technique; MDT: Microdilution technique; SC2: Pent-4-enoate

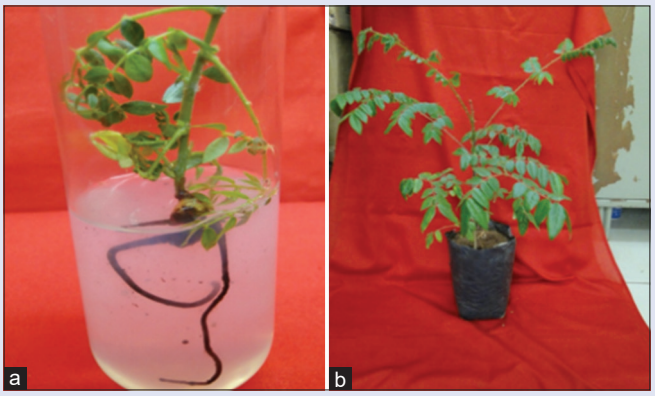


Figure 4: Rooting of the organized shoot and soil acclimatized plantlets: (a) Growth of shoot with serrate margined leaves and rooting on half-strength MS medium and (b) Hardened potted plant

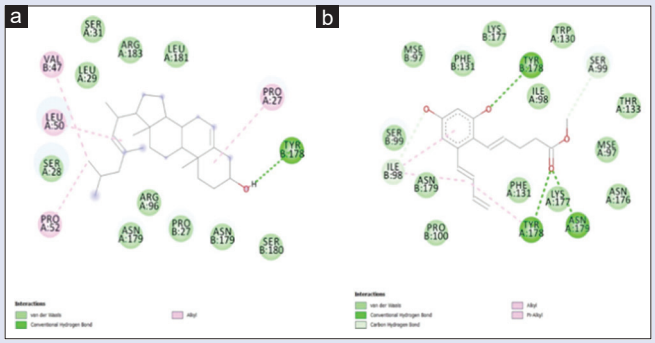


Figure 5: (a) The 2D Discovery Studio analysis and docking results showing the interaction between LC3 and 2H12; and (b) The 2D Discovery Studio analysis and docking results showing the interaction between SC2 and 2H12

Many woody legume species are propagated only through the seeds.[19] Loss of viable germplasm, extensive dormancy period, and destruction of habitat resulted in the depletion of the population. Therefore, the development of a protocol for regenerative potentialities is of utmost importance for the conservation of the woody legumes.[20,21]

Various explants, namely flower bud, flower stalk, leaf tip, leaf with midrib, leaf blade, and petiole were cultured for callus induction and *in vitro* development of plantlets.[21] The age of the flower bud was found to be an important factor in callus induction and shoot proliferation. There are some previous reports on using of flower bud as explants for shoot regeneration.[22] In the present stud, *C. bonducella* floral callus was initiated on the media supplemented with a range of 0.5–3 mg/l 2, 4-D, and 0.1–1 mg/l BAP. Callogenesis was initiated from the thalamus region of the flower, gradually it proceeded toward the nonaccessory and accessory floral parts of the flower. Optimal callogenesis was noticed from the flower bud explants at the concentration of 0.5 mg/l BAP and 2 mg/l 2, 4-D. The primary calli subcultured on to the shooting media which contain MS basal salts supplemented with a range of 2–5 mg/l BAP and 0.3–0.9 IBA for shoot induction and high-frequency shoot induction was noticed at 3 mg/l BAP and 0.5 IBA (3.80 ± 0.79). After 4 weeks of shoot initiation, the shootlets grew above 3–4 cm length were transferred to rooting media augmented with different concentrations of IBA (0.1–1.0 mg/l). The regenerants were hardened primarily in the polythene bags at greenhouse condition and transferred to the pots containing sterilized soil. The regenerants are successfully hardened in outside the laboratory. Through this investigation, it has been possible to develop an efficient and reproducible *in vitro* mass propagation system from flower buds of *C. bonducella*.

The isolates of *C. bonducella* were tested for their *in silico* antibacterial activity against *L. interrogans*. Thus, the *in silico* analysis became the supporting data as it was reported that *C. bonducella* exhibits a good antibacterial inhibition activity. By this, our investigations on *C. bonducella* stood apart from the previous work carried out on the same plant. In the present study, the molecular docking of two phytoconstituents LC3 and SC2 against citrate synthase and LipL32 proved the antileptospiral activity of *C. bonducella,* by forming a good amount of bonded (hydrogen bonds) and non-bonded interactions (hydrophobic interactions) and also exhibited higher binding affinity with the receptors. The *in silico* studies suggested that both LC3 and SC2 showed a remarkable antibacterial effect by inhibiting the bacterial citrate Synthase and LipL32.

Billah *et al*., 2013, have shown the activity of crude methanol extract of *C. bonducella* leaves possess antibacterial, antidiarrheal, and cytotoxic activities and the experiments conducted by Subbiah *et al*., 2019, showed the inhibition of α-amylase activity, which showed that the plant may be used in antidiabetic therapy. Further, they reported the antidiabetic, anti-inflammatory, antioxidant, antimicrobial, and antimitotic activity. The study conducted by Shirish *et al*., 2017, investigated the *in vitro* antibacterial activity of the extracts of *C. bonducella* leaves extracts.[23-27]

CONCLUSION

The *in silico* molecular docking and *in vitro* approach were carried out with LC3 and SC2 to check the antileptospiral activity. The

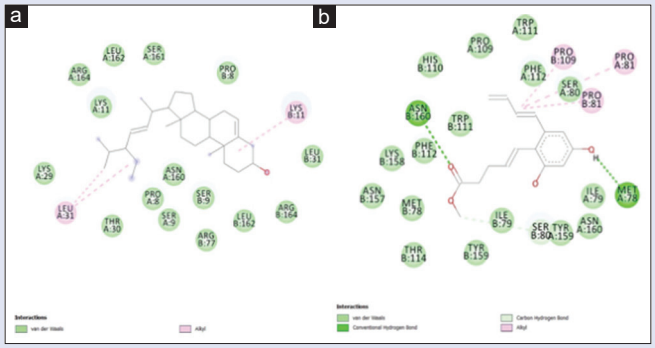


Figure 6: (a) The 2D Discovery Studio analysis and docking results showing the interaction between LC3 and 2ZZ8; and (b) The 2D Discovery Studio analysis and docking results showing the interaction between SC2 and 2ZZ8

methyl (4*E*)-5-{2-[(1*E*)-buta-1,3-dien-1-yl]-4,6-dihydroxyphenyl} pent-4-enoate when compared to β-Sitosterol has shown a remarkable anti-leptospiral activity against the *L. interrogans* through the inhibition of citrate synthase and LipL32.

Acknowledgements

The authors are grateful to the financial assistance from DBT-BUILDER Program, New Delhi, India, and authors acknowledge the support and infrastructure provided by the Davangere University, Davangere, JSS Academy of Higher Education and Research, Mysuru, India, and the Director, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru for infrastructure support. The author extends their appreciation to The Researchers Supporting Project number (RSP-2020/15) King Saud University, Riyadh, Saudi Arabia.

Financial support and sponsorship

The authors are thankful to DBT, New Delhi, India, for providing financial support through the DBT-BUILDER program (Order No. BT/PR9128/INF/22/190/2013, Dated: 30/06/2015) and the Kuvempu University administrative authority for offering the facility to carry out the work. The authors are grateful to the financial assistance from DUSMYTR- GRANT SANCTION LETTER , No: DU/HRM/2020-21/6045/DATED:03-03-2021.Davangere University, Davangere, Karnataka India and Authors acknowledge the support and infrastructure provided by the Davangere University, Davangere, Karnataka, India.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Handa SS, Maharaj KK. Supplement to Cultivation and Utilization of Medicinal Plants. Jammu Tawi: Regional Research Laboratory, Council of Scientific and Industrial Research; 1996.
2. Kapoor LD, Handbook of Ayurvedic Medicinal Plants, Herbal Reference Library, 1st edition, CRC Press (ISBN 9780849329296), 2000.
3. Elizabeth M Williamson, Major herbs of Ayurveda. Edinburgh ; New York : Churchill Livingstone, 2002.
4. Kirtikar, Ranchoddas K. Indian Medicinal Plants. Allahabad: Lalit Mohan Basu; 1935.
5. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems. Biochemistry 1981;20:6006-12.
6. Cheruvathur MK, Britto J, Thomas TD. Callus induction and shoot regeneration from epicotyl explants of ethnomedicinally important *Caesalpinia bonduc* (L.) Roxb. Iran J Biotechnol 2010;8:22-9.
7. Umesha S, Chandan S, Gopinath SM, Shareef MI. Detection of *Leptospira* in canine samples using polymerase chain reaction based on lipl32 gene and biochemical profiles in canine leptospirosis. J Cell Sci Mol Biol 2014;1:105.
8. Prasad SK, Pradeep S, Shimavallu C, Kollur SP, Syed A, Marraiki N, *et al.* Evaluation of Annona muricata Acetogenins as Potential Anti-SARS-CoV-2 Agents Through Computational Approaches. Front. Chem 2021;8:624716.
9. Kollur SP, Prasad SK, Pradeep S, Veerapur R, Patil SS, Amachawadi RG, *et al.* Luteolin-Fabricated ZnO Nanostructures Showed PLK-1 Mediated Anti-Breast Cancer Activity. Biomolecules 2021;11:385.
10. Jain AS, Sushma P, Dharmashekar C, Beelagi MS, Prasad SK, Shivamallu C, *et al. In silico* evaluation of flavonoids as effective antiviral agents on the spike glycoprotein of SARS-CoV-2. Saudi J Biol Sci 2021;28:1040-51.
11. Prasad A, Shruthi G, Sushma P, Jain AS, Chandan D, Prasad MN, *et al. Helicobacter pylori* infection: A bioinformatic approach. Int J Pharma Sci Res 2020;11:5469-83.
12. Avinash KO, Sushma P, Chandan S, Gopenath TS, Kiran MN, Kanthesh BM, *et al.* In Silico Screened Flavanoids of Glycyrrhiza glabra Inhibit Cpla2 And Spla2 In Lps Stimulated Macrophages. Bull. Env. Pharmacol. Life Sciences 2021;10:14-24.
13. Uppar V, Chandrashekharappa S, Shivamallu C, Pradeep S, Kollur SP, Ortega-Castro J, F*et al.* Investigation of Antifungal Propertiesof Synthetic Dimethyl-4-Bromo-1-(Substituted Benzoyl) Pyrrolo[1,2-a]Quinoline-2,3-DicarboxylatesAnalogues: Molecular DockingStudies and Conceptual DFT-BasedChemical Reactivity Descriptors andPharmacokinetics Evaluation. Molecules 2021;26:2722.
14. Govindaraju S, Vinayagamurthy B, Prasad S, Swamy N, Chandan S. Fatty acid metabolism in *Leptospira* a key to its pathogenicity and evasion from host immune response leading to prolonged survival of the organism. Indian J Nat Sci 2017;7:0976-97.
15. Ankegowda VM, Kollur SP, Prasad SK, Pradeep S, Dhramashekara C, Jain AS, *et al.* Phyto-mediated synthesis of silver nanoparticles using *Terminalia chebula* fruit extract and evaluation of its cytotoxic and antimicrobial potential. Molecules 2020;25:5042.
16. Khalil S, Cheah K, Perez E, Gaskill D, Hu J. Regeneration of banana (*Musa* spp. AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. Plant Cell Rep 2002;20:1128-34.
17. Akter N, Hoque MI, Sarker RH. *In vitro* propagation in three varieties of gerbera (*Gerbera jamesonii* Bolus.) from flower bud and flower stalk explants. Plant Tissue Cult Biotechnol 2013;22:143-52.
18. Kumar PA, Tarun K. Efficient micropropagation and evaluation of genetic fidelity of *in vitro* raised plants of *Commiphora wightii* are. (Bhandari) – A medicinally important red-listed species of arid regions. J Plant Dev 2012;19:29-40.
19. Murgai P. *In vitro* culture of the inflorescences, flowers and ovaries of an apomict, *Aerva tomentosa* Forsk. Nature 1959;184:72-3.
20. Motiur R, Bulbul A, Rafiul I, Abul M, Monzur H. A biotechnological approach for the production of red gerbera (*Gerbera jamesonii* Bolus). Nova J Med Biolo Sci 2014;2:1-6.
21. Nagarathnamma M, Sudarshana, MS, Niranjan MH. Plant regeneration from inflorescence cultures of *Gynandropsis pentaphylla* DC. J Appl Pharm Sci 2013;3:153-5.
22. Jiliang P, Lilin W, Taihe X, Dan Z, Hong Y. High-frequency floral bud regeneration from petal segment cultures of *Sinningia speciosa* Hiern. Chin J Cell Biol 2012;34:279-85.
23. Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SA, *et al.* Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonducella* (L.) Roxb leaves. BMC Complement Altern Med 2013;13:101.
24. Subbiah V, Nagaraja P, Narayan P, Nagendra HG. Evaluation of pharmacological properties of *Caesalpinia bonducella* Seed and Shell Extract. Pharmacog J 2019;11:150-4.
25. Khan N, Sunil K, Pal SR, Neelam D. A pharmacognostic and pharmacological overview on *Caesalpinia bonducella*. Res J Pharm Biol Chem Sci 2012;3:440-96.
26. Shirish SP, Manohar GC, Nirmala RK. Phytochemical analysis and antibacterial activity of *Caesalpinia bonducella* leaves. Int J Pharm Sci Rev Res 2017;42:217-20.
27. Shukla S, Mehta A, Mehta P, Vyas SP, Shukla S, Bajpai VK. Studies on anti-inflammatory, antipyretic and analgesic properties of *Caesalpinia bonducella* F. seed oil in experimental animal models. Food Chem Toxicol 2010;48:61-4.