# Conclusion

The present study provided scientific evidence of the therapeutic efficiencies of *C. bonplandianus* which were claimed in the ethnopharmacological domain. The entire work was performed with 70% hydromethanolic extract of *C. bonplandianus* because this extraction process is generally considered one of the best method for extraction of the phenolic and flavonoid compounds, which are known responsible for bioactivities of any herbal medicine. Moreover, in ethnopharmacology, the majority of the medicines are actually tinctures, prepared through similar hydroalcoholic solvent extraction process.

Initial screening of CBLE was performed through the evaluation of their antioxidant and free radical scavenging activities. At first, the overall antioxidant capacity was confirmed by DPPH ($IC_{50}$: 3.79±0.06 µg/ml) assay, which revealed satisfactory bioactivities of the extracts. Thereafter, selective free radical scavenging assays were performed against different free radicals to find out whether the extracts could demonstrate inhibitory activity on individual free radicals. CBLE in case of OH• ($IC_{50}$: 73.65±4.96 µg/ml) and NO ($IC_{50}$: 36.74±2.79 µg/ml) and in case of HOCL ($IC_{50}$: 66.58±4.39 µg/ml) scavenging activity demonstrated better efficacy than the respective standards ($IC_{50}$: OH• (mannitol) 571.45±20.12 µg/ml; NO (curcumin) 90.82±4.75 µg/ml; HOCL (ascorbic acid) 235.96±5.75 µg/ml). The results of the free radical scavenging assays were convincing, which led us to evaluate possible in vitro lipid peroxidation inhibitory ($IC_{50}$: 19.70±1.32 µg/ml) and iron chelation ($IC_{50}$: 123.46±1.92 µg/ml) activities of the extracts. Total phenolic and flavonoid contents were also estimated in parallel to the antioxidant assays as the antioxidant capacity of any herbal formulations rely mostly on the load of both classes of phytocompounds. On later part of this study, the protective roles of *C. bonplandianus* through its antioxidative properties were also

demonstrated under *in vivo* neuromodulatory and hepatoprotective evaluations.

The assessment of immunomodulatory activities of *C. bonplandianus* was divided into two broad sections i.e. the overall immunomodulatory activities and anti-inflammatory activities. The assays were performed both through *in vitro* and *in vivo* methods. The effect of CBLE on the murine humoral response was evaluated through PFC, HA titre assay and subsequent estimation of IgM levels in serum samples. CBLE resulted in significant stimulation of the humoral immune system. The extract was in parallel investigated for their efficiency to modulate the effector functions of the macrophages, isolated from the murine peritoneal cavity. Elevation in the phagocytic capacity was observed in case of CBLE. However, mild increases in macrophage population (1.69, 1.05 and 1.20 fold) were observed for all the extracts. The extracts further demonstrated modulation of cell adhesion property and phagocytosis related respiratory burst (1.70, 1.32 and 1.31 fold increase) and MPO release under *in vitro* condition. Majority of the anti-inflammatory study was performed on *in vitro* murine splenic lymphocytes which were stimulated with optimum dose (5 μg/ml) of Con A. Quantification of different pro-and anti-inflammatory markers demonstrated the efficiency of *C. bonplandianus* extracts to down-regulate TNF-α and IL-4 in addition up-regulate IL-2, IL-10 and IFN-γ levels. The release of NO from lymphocytes and macrophages demonstrated inhibitory activity of the extracts. The anti-inflammatory activities were further established through inhibition of COX-1 (0.48, 0.80, 0.84 fold), COX-2 (0.39, 0.55, 0.68 fold) activities and associated $PGE_2$ release from the lymphocytes.

Under hepatoprotective evaluation, hepatic injury to murine liver was introduced through oral administration of a potent haloalkane hepatotoxin $CCl_4$ and subsequently treated with *C. bonplandianus* extracts. Biochemical analysis of the serum samples from the experimental animals revealed decrease of various liver marker enzymes and biochemical parameters such as ALT (0.51, 0.64 and 0.75 fold), AST (0.57, 0.72 and 0.80 fold), ACP (0.66, 0.79 and 0.78 fold), ALP (0.49, 0.78 and 0.77 fold), GGT (0.53, 0.80 and 0.73 fold), bilirubin (0.53, 0.78 and 0.87 fold) etc. in the extract treated groups. Outcomes of the *in vitro* results were

also in accordance with the *in vivo* studies performed through liver explant cultures. Hepatic catalyse (0.52 fold) and peroxidase (0.48 fold) activities, which were significantly lowered due to $CCl_4$ toxicity, were found to be up-regulated (catalase: 1.52, 1.27 and 1.26 fold; peroxidase: 1.21, 1.18 and 1.22 fold) due to extract treatment. This also correlated with the lowering of lipid peroxidation (0.56, 0.79 and 0.78 fold). Two major hepato-inflammatory markers i.e. NO (0.19, 0.44 and 0.50 fold) and TNF-α (0.14, 0.28 and 0.38 fold) were also found to be lowered in case of extract treated groups. Furthermore, histopathological studies of the liver samples provided evidence for lowering of hepatic inflammation, fatty infiltration, bile duct proliferation, hepatocellular necrosis, calcification, sinusoidal dilatation etc. in the extract treated groups. Molecular docking analyses revealed that the compound α-amyrin present in leaf extract of *C. bonplandianus* has the potent hepatoprotective role better than the standard molecule silymarin.

The photochemical composition of leaf of *C. bonplandianus* was investigated initially through biochemical spectrophotometric methods, which revealed the presence of alkaloid, saponin, polyphenols, flavonoids and vitamins. CBLE were further subjected to FTIR analysis which revealed the major chemical groups (alcohol, amide, alkane, nitro, acid, ketone etc.) in the extract. Further bi-fractionation of the extracts using DCM and n-hexane and subsequent GC MS analysis revealed the presence of different bioactive phytocompounds (vanillin, phytol, vaccenic acid, vitamin E, stigmasterol, β-sitosterol, α-amyrin etc.).

**The major findings of the present study may thus, be summarized as follows:**

- *C. bonplandianus* possess potent antioxidant activities as demonstrated by DPPH, total antioxidant activity and reducing power activities.
- Free radical scavenging potentialities of *C. bonplandianus* was established through inhibition of the oxygen and nitrogen free radicals, which would further prove beneficial in attenuation of oxidative stress in various diseases.
- *In vivo* immunomodulatory studies revealed stimulation of humoral immunity by *C. bonplandianus*.

- *C. bonplandianus* demonstrated profound influence on macrophage activities.
- Modulation of cytokine levels along with inhibition of COX activities, $PGE_2$ and NO levels would provide protection in inflammatory conditions.
- Normalization of liver biochemical and enzymatic markers may provide beneficial under chronic hepatic damage.
- Inhibition of NO and TNF-α associated lowering of liver injury scores would prove beneficial in hepatic inflammation.
- Elevated catalase and peroxidase activities along with the inhibition of lipid peroxidation would prove beneficial under hepatic damage and neuromodulation.
- Bioactivity of *C. bonplandianus* leaf was found higher than that of stem and root in most cases.
- The various phytocompounds identified through FTIR and GC -MS analysis, are responsible for the potent bioactivities of *C. bonplandianus*.
- α-amyrin, the compound indentified by GC-MS analyses present in the leaf extract of *C. bonplandianus* has the potent hepatoprotective activity than the standard silymarin.