ABSTRACT

Higher plants are a rich source of bioactive constituents or phyto-pharmaceuticals used in the pharmaceutical industry. Many of these pharmaceuticals are still in use today and are often used as synthetic substitutes that have been found to possess the same efficacy and pharmacological specificity. Phytochemicals of only a small percentage have been studied and of these phytochemicals, an even lesser percentage has been examined for their biological properties as its research involves a complex and expensive process in which research has plenty of space to improve steps to utilize the biologically active compounds from plant resources. *Indigofera caerulea* Roxb is a well known shrub among native medical practitioners in folk medicine and is used for the treatment of jaundice. Studies on the systemic research methodology and scientific evaluation based on traditional herbal medicines of *I. caerulea* remain unexplored. The present study dwells on the identification, isolation and *in silico* analysis of bioactive compounds from *I. caerulea* through various biological and chromatographic techniques based on the activity guided fractionation of its leaf methanolic extract.

Total phenol and antioxidant activity of different solvent extracts of *I. caerulea* leaves were investigated. Extraction was done sequentially in soxhlet apparatus using various solvents (Petroleum ether, ethyl acetate and methanol). Antioxidant activity was evaluated by 2, 2-diphenyl-1-picryl hydrazyl free radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay and total ion reducing power assay. Hepatoprotective effect of the methanolic extract of *I. caerulea* leaves (MIL) and elucidation of its mode of action against carbon tetrachloride (CCl₄) induced liver injury in rats were demonstrated. HPLC analysis of MIL when carried out showed peaks close to standard ferulic acid and quercetin. Intragastric administration of MIL up to 2000 mg/kg bw, did not show any toxicity and mortality in acute toxicity studies. Investigation of the underlying
mechanism revealed that MIL treatment was capable of reducing inflammation by an antioxidant defence mechanism that blocks the activation of NF-κB as well as inhibits the release of proinflammatory cytokine TNF-α and IL-1β.

Further chromatographic and spectroscopic led isolated compounds were characterized and their assumed structures were as follows. The probable structure of compound MIL-1 (F7) was identified as long hydrocarbon chain with a carbonyl group and a hydroxyl function. Fraction MIL-2 (F10) was identified as ethyl linoleate and its molecular formula: C_{20}H_{36}O_{2}, molecular weight: 308.498646 g/mol also obtained. Fraction F12 was identified as fructose Molecule. The compounds such as ferulic acid and quercetin identified by RP-HPLC along with ethyl linoleate (Spectroscopic led identified) were also selected for molecular docking studies against TNF-α (2AZ5) and COX-2 (3LN1) inflammatory mediator proteins. RP-HPLC identified small molecules with chromatographic identification and isolated ligand compounds which were utilized in molecular docking interaction studies and proved to be effective against pro inflammatory mediators. Molecular dynamics (MD) simulation studies of TNF-α-quercetin complex (20 ns) also provides the strong evidence for the inhibitory effect of quercetin against TNF-α. The mechanism of action of *I. caerulea* in inflammatory liver ailments was hypothesize in both *in vivo* and *in silico* studies. The study altogether provides insight on the phytochemical profile of *I. caerulea* and justifies the use of this plant in traditional medicine for the treatment of jaundice and could be of great importance for the treatment of oxidative damage and free radical related diseases. This is the first scientific report on the *I. caerulea*.