Abstract

Herbals are the core of traditional medicine. Several plants have been identified and extensively utilized for their therapeutic value from the very beginning of civilization. More than 3000 plants species from 200 families were identified to have medicinal properties in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island of India. *Clerodendrum* is a member of Lamiaceae family; mostly they are perennial shrubs and in Indian Ayurvedic system they are used in many of the herbal preparations. Species of *Clerodendrum* are distributed in tropical regions of Asia including India, Myanmar, Bangladesh, Malayasia, Indonesia, Thailand, Bhutan, Nepal and also in temperate Tibet. Moreover, the medicinal properties of the species are attributed to their phytochemical constituents that are believed to cure varied ailments. Although, ethnobotanically, they have diverse medicinal properties but their experimental validations are largely incomprehensible. Therefore, the present study was conducted to evaluate some of the medicinal properties and related pharmacological potentialities of selected members of the genus *Clerodendrum*. Initial screening was done by evaluating the *in-vitro* antioxidant and antimicrobial activities. The potent hepatoprotective potentiality was studied on haloalkane induced acute hepatic damage in mouse. The acclaimed neuromodulatory activity of *C. serratum* was investigated on scopolamine induced murine model. In -depth nephrotoxicity study was also conducted on rat model. A detailed phytochemical analysis was also performed to elucidate the major bioactive species in the plant. In addition, an attempt was made to access the genetic relationship among the 11 species of *Clerodendrum*.

In the antioxidant profiling, all the *Clerodendrum* species (CIL, VIL, CSL and CCL) exhibited higher free radical scavenging activity than the respective standard (ascorbic acid) as per DPPH assay. Among the four extracts, the CCL (65.78±1.04 % at 200µg/ml) extract showed higher percent of

inhibition when compared to standard (58.5±0.02 % at 200µg/ml). All the extracts of *Clerodendrum* species (CIL, VIL, CSL and CCL) were found to exhibit moderate scavenging activity in case of superoxide anion, hypochlorous acid, nitric oxide and lipid peroxidation scavenging assay in a dose dependent manner and highly significant scavenging activity in case of hydroxyl radical, peroxinitrate and hydrogen peroxide scavenging assay. Among the four species of *Clerodendrum*, CCL contained highest phenolic and flavonoid content. These contents exert crucial antioxidant scavenging activities in all the plants which clearly display potent medicinal activity.

To investigate the probable cytotoxic effects of extracts, a good deal of experiments were performed. In brief, significant percentage of erythrocyte membrane protection was detected in all of four extracts while negligible amount of haemolytic effect towards human erythrocytes was observed in CIL, VIL, CSL and CCL extract indicating non-detrimental effect on erythrocytes. Furthermore, MTT cell viability test on splenocyte cells revealed no cytotoxic effect on splenocyte cells during the exposure of highest dose. Therefore, all the extracts could be well treated as consumable bio -safety stuff, making them suitable for the preparation of drugs involved in the treatment of various diseases.

The methanolic extract of four *Clerodendrum* species namely *C. indicum*, *C. inerme* (syn: *Volkameria inermis*), *C. serratum* and *C. colebrookianum* showed antibacterial activity against two pathogenic gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and two pathogenic gram negative bacteria *Escherichia coli* and *Enterobacter aerogenes* at a concentration of 0.1, 0.25, 0.5 and 1.0 mg/ml. The findings in the present study offer a scientific support to the use of leaves of four *Clerodendrum* species as a new antibacterial drug against bacterial infection in future.

Based on the ethnopharmacological claims, hepatoprotective potentialities of *C. indicum*, *V. inermis* and *C. colebrookianum* were studied in CCl_4 induced murine model. The result demonstrated the significant inhibition of catalase, peroxidase, SOD (superoxide dismutase) and reduced glutathione (GSH) by *Clerodendrum* (CIL, VIL and CCL) extracts occurred in CCl_4 intoxicated mice when compared with the control. The MDA

content was elevated from 8.98 ± 1.56 mM/litre in control to 19.27 ± 1.31 mM/litre in CCl_4 group. Significant results found when the elevated MDA level was lowered to 5.64 ± 0.86 mM/ litre after CCLH administration. However, significant ($p < 0.001$) lowering of NO level was observed in the treated groups. The NO level in silymarin and CCLH groups were 142.37 ± 11.70 and 175.33 ± 12.50 % respectively when NO release of control was considered as 100 %. The detailed histopathological study also supports the *in-vivo* hepatoprotective activity. In this study, it is established that the diminished catalase, peroxidase and superoxide dismutase levels and elevated MDA levels were subsequently normalized by *Clerodendrum* administration.

Clerodendrum serratum demonstrated neuromodulatory activity by the inhibition of *in-vivo* acetylcholinesterase, DPPH, catalase, GSH and SOD activities. The extent of inhibition of lipid peroxidation was significant in leaf extract. The neurotherapeutic effect of CSL on memory deficits in a mouse model of amnesia (passive avoidance test) induced by scopolamine was evaluated. As a result, passive avoidance test , a fear motivated avoidance test, was employed to describe the way in which the animal learn to avoid an aversive stimulus (electric foot shock) as a part of long term memory. The histological study and *in-silico* study also confirmed that this plant extract is promising in elevating neurodegenerative disorders. Therefore, it seems likely that this plant can be used as anti-cognitive agent for future drug development in Alzheimer's and Parkinson's diseases.

What's more, *C. serratum*, an important medicinal member of Lamiaceae, was found to be used as a traditional nephrotoxic remedy in Bangladesh, Pakistan, Egypt, Nigeria and in Ayurveda as well. CSL extract showed promising result in all *in-vivo* enzymatic assays (Superoxide dismutase, Glutathione reductase, Lipid peroxidation and catalase) and *in -vitro* biochemical assays (creatinine and urea). The histological slides and *in-silico* study confirmed that *C. serratum* extract is promising in elevating nephrotoxicity in rat model.

Since the extracts exhibited potent antioxidant, hepatoprotective, anti-neurodegenerative and nephrotoxic activity, it would be amicable for one to identify the active phytocompounds

responsible for those activities present in the extracts. In this regard, FTIR and GC-MS analysis have been considered. The IR spectrum of CIL, VIL and CCL extracts indicated the presence of major peaks like amines, ether, alcohol and carboxylic acid at 1081 cm^{-1}, 1245 cm^{-1}, 3294 cm^{-1}, 1734 cm^{-1} respectively. The present study was extended for the analysis pertaining to the identification of active compounds in CIL, VIL, CSL and CCL using GC-MS method. A total number of forty five (45) phytocompounds have been identified in CIL (10 compounds), VIL (14 compounds), CSL (12 compounds) and CCL (10 compounds). Among the compounds, linoleic acid (LA), dodecanoic acid and hexadecanoic acid are some of the essential fatty acids that human being requires in diet. In addition, squalene and stigmasterol have been reported to be potent antioxidants and observed to be effective against several oxidative stress related diseases. Hence from the above illustration, it might be inferred that CIL, VIL, CSL and CCL extract could be regarded as a potent future antioxidative agents.

To understand better activities at a molecular level an *in-silico* approach was adopted. A few essential proteins deeply involved in internal antioxidant machinery as well as having implications in cell growth and proliferation cascades were thoroughly examined in a docking environment, where they were checked against the phytochemicals (ligands) present in our studied plants. The selected protein (Nrf2, Ap1 and p53) generally plays a vital role in cell division, development, apoptosis, tumor suppression and natural antioxidant properties. 24, 25-Dihydroxyvitamin D of *C. indicum* (CIL), Ethyl iso allocholate of *V. inermis* (VIL) and Stigmasterol of *C. colebrookianum, C. serratum* (CCL and CSL) showed higher binding affinity compared to other compounds. Among them on an average stigmasterol was found to have a slight upper hand compared to the remaining compounds. However, when individual interactions were compared, 24, 25-Dihydroxyvitamin D displayed the highest binding affinity with Nrf2 protein.

The bioactive compounds of CIL, VIL and CCL were checked for possible interactions with several proteins playing the essential role in different metabolic pathways of humans and other major vertebrates. The proteins were chosen those have relationship

with the health of the liver. These proteins acted as receptors required for molecular docking experiments. The highest binding affinity was found between Stigmasterol and a protein with PDB ID 3i7h which is the crystal structure of DDB1 in complex with H-Box Motif of HBX. In addition, decent binding pattern was also admired between Stigmasterol and human brain membrane protein (dopamine receptor D3 protein; ID- 3PBL) claiming their strong function in the management of neurodegeneration like Alzheimer's disease, Perkinson's disease etc. Furthermore, successful binding between Stigmasterol and Polycystic kidney disease protein 1-like 2 (PKD2) demonstrates the best results with a binding affinity of -8.6 kcal/mol in autodock vina. Hence, present finding could open a new door to understand the roots of several diseases and disorders facilitating new drug discovery.

After getting noteworthy results in all aspects of experimentation expressing its potent therapeutic activities, we focused on the isolation of active compounds from the leaves of *V. inermis* (VIL) using column chromatography. The obtained fractions (fraction-1 and fraction-2) were elucidated by spectroscopic methods including IR, ^{1}H-NMR and ^{13}C-NMR. After structure elucidation various biological activity such as antioxidant and antimicrobial activity of the isolated compounds were performed. The recorded IR and NMR spectrum of fraction-1 and fraction-2 was compared with the literature spectral data and its quiet similar to the spectra of Squalene and 9, 12, 15-Octadecatrienoic acid, methyl ester (linolenic acid methyl ester). The compounds also showed significant antioxidant and antimicrobial activity.

In this study, *in-vitro* callus regeneration of *C. thomsoniae* through nodal culture has been attempted. Murashige and Skoog's medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA was found to be most effective in callus induction (85%). MS with BAP was found suitable for shoot and root development. To detect somaclonal fidelity in *C. thomsoniae,* RAPD and ISSR markers were employed. Ten RAPD decamers produced 65 amplicons, while ten ISSR primers generated 75 bands in both *in-vitro* plantlets and field grown plants. The amplified products of parent plants and the regenerated plants were found to be monomorphic in RAPD and ISSR

analyses. A number of compounds with potential therapeutic and biological activity had been detected with the help of GC-MS analysis. Taking into account of all the GC-MS and *in-silico* molecular docking data, it can be concluded that some of these compounds may hold promise as medically relevant candidates and might prove beneficial in future drug development.

Apart from medicinal aspect, no comprehensive attempt was carried out to explore the genetic variations among 11 different species of *Clerodendrum*. Hence, an initiative was undertaken to discriminate the genetic variations among 11 important species of *Clerodendrum* through DNA fingerprinting techniques. A good deal of genetic diversity was recorded in the present study. For instance, a total of 495 polymorphic bands were generated during RAPD analysis ranging from 124 bp to 1980 bp with 100% of polymorphism using 45 primers while in case of ISSR analysis a total of 15 primers were used and all the primers showed positive response to generate distinct, scorable bands. A total of 229 amplified bands were produced by the 15 primers of which all the 229 were polymorphic. The frequency of polymorphism was therefore found to be 100%. The band size ranged between 111 bp to 1350 bp. Whereas, PCR-RFLP analysis accounted a total of 8, 4 and 12 polymorphic bands with 100%, 50% and 70.58% of polymorphism in case of matK, Rps16 and trnL-trnF region of the chloroplast genome respectively. It may be presumed that this polymorphism was resulted due to their polyphyletic nature of 11 species under the genus *Clerodendrum.* Further DNA barcode analysis by means of matK, Rps16 and TrnL-F clearly reflected that two subfamily Symphorematoideae and Nepetoideae very close to Ajugoideae which validates the traditional classification of Cronquist.