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*Research Article*

# *In-silico* molecular docking study of phytochemicals obtained from *Clerodendron colebrookianum* for hepatoprotective potentialities

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## Abstract

Herbs are the core of traditional medicine. Several plants have been identified and extensively utilized for their therapeutic value from the very beginning of civilization. *Clerodendrum colebrookianum* 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. *C. colebrookianum* are distributed in tropical regions of Asia including India, Myanmar, Bangladesh, Malayasia, Indonesia, Thailand, Bhutan, Nepal and also in temperate Tibet. Since the extracts exhibited potent hepatoprotective, nephroprotective and anti-hypertensive activity, it would be amicable to identify the active phytocompounds responsible for those activities present in the extracts. In this regard, GC-MS analysis has been considered. The A total number of six (6) phytocompounds have been identified in *C. colebrookianum* leaves (CCL). Among the compounds, Linolenic acid, methyl ester acid and hexadecanoic acid are some of the essential fatty acids that human being requires in diet. The bioactive compounds of 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 (-8.7 kcal/mol) 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. Hence, present finding could open a new door to understand the roots of several diseases and disorders facilitating new drug discovery.

## 1. Introduction

India, one of the richest floristic regions of the world, has diverse socioeconomic, ethnic, linguistic and cultural areas. There are about 54 million indigenous people of different ethnic groups colonizing various regions of the country. The aboriginal groups have their own distinctive culture, religious rites, food habit and a rich knowledge of plant utilization [1,2] which pass orally generation to generation. Therefore, the traditional knowledge of medicinal plants and their use in treating several ailments might reasonably be expected in India due to its rich floristic vegetation [3]. Chandel *et al*. [4] have reported that nearly 70% of tribal and rural inhabitants of India are to a large extent depended on medicinal plants for their primary healthcare management due to either insufficient or inaccessible or less availability of modern healthcare system. Virtually, ethnobotanical survey may be regarded as one of the most reliable approaches towards new drug discovery and it is a prerequisite for any developmental planning concerned with the

welfare of tribal and their environment [5]. Nonetheless, in recent times medicinal plants became the backbone of herbal drugs being used over the world wide.

*Clerodendron colebrookianum* is a woody or semi woody shrub, 3.7-4 m long, belonging to the family Lamiaceae [6]. Ethnomedicinally, different parts including leaf, stem and root extracts have been reported to have several medicinal properties including rheumatism, asthma, malaria, anti-diabetic, hepatoprotective, nephroprotective and anti-hypertensive [6]. In fact, this species is commonly used by ethnic people in various parts of North-East India as a source of polyherbal formulations for the treatment of various diseases [7,8]. Ethnobotanically *C. colebrookianum* have diverse medicinal properties but their experimental validation is largely incomprehensible. Therefore, the present investigation aims for an in-depth in-silico analysis about hepatoprotective potentialities of *C. colebrookianum*.

## 2. Materials and Methods

### *2.1. Plant material collection and extract preparation*

*Clerodendrum colebrookianum* leaves (CCL) were collected from Guwahati, Assam (26.1445° N, 91.7362° E). The plant material was identified by plant taxonomist and the voucher specimen (Accn. # CCL/NBU/09816) was deposited at the Herbarium of the Botany department. Air-dried (3 weeks) fresh leaves of *C. colebrookianum* (25 g) were pulverized into fine powder by using mechanical grinder. The powdered leaves of *C. colebrookianum* (10 g) were extracted in a Soxhlet apparatus using absolute methanol (the ratio of plant material to solvent was 1:10 m/v) for 6-7 hours. The extract was then concentrated under reduced pressure and controlled temperature (40°-50 °C) using rotary evaporator (Buchi Rotavapor R-3, Switzerland). The extract was further lyophilized using Eyela Freeze Dryer (FDU-506, USA) to obtain dry powder and stored at 4°C until required. The lyophilized CCL extract was dissolved in absolute methanol in desired concentrations each time just prior to use.

### *2.2. GC-MS analysis*

In split mode, one microliter of the sample was injected into a GCMS-QP2010 Plus unit. Temperatures including the injection, interface, and ion source temperatures were set to 260 °C, 270 °C and 230 °C, respectively. Helium was used as a carrier gas while the total flow and the column flow rate were 16.3 mL/min and 1.21 ml/min, respectively. Mass spectra were recorded at 5 scans/s with a scanning range of 40-650 m/z. Compounds were quantified by analyzing the peak areas, and this was further validated based on the available literature [9].

### 2.3. *In-silico* molecular docking

Following successful characterization of phytochemicals from *C. colebrookianum* extract, *in-silico* docking techniques towards the discovery of potent drug was undertaken to establish whether the phytochemicals have any role in the management of different imperative diseases including hepatoprotective.

#### *2.3.1. Preparation and refinement of the protein and ligand structures*

The chemical structures for the identified phytochemicals were downloaded in .sdf format from NCBI-PUBCHEM (http://www.ncbi.nlm.nih.gothrv/pccompound) followed by converted into .pdb format through SMILES server (https://cactus.nci.nih.gov/translate/). The PDB protein structures were prepared for docking after deletion of water atoms followed by addition of polar hydrogen atoms. Gasteiger charges were further added to both proteins and ligands on the basis of electronegativity equilibration. The torsions of all ligands (i.e. selected phytocompounds) were allowed to rotate freely to obtain better outcome. Molecular docking was performed by Autodock Vina and the docked complexes were visualized by means of PyMol protein structure visualization package through which the relative distance between the atoms of ligands and proteins were also generated [in the unit of Angstrom (Å)].

#### *2.3.2. Molecular docking and hepatotoxic activity*

Proteins were chosen based on literature survey, having functional implications in hepatotoxic activity. The receptor structures were defined as rigid, and the grid dimensions were 126, 126 and 126 for the X, Y, and Z axes for protein NF-κβ having PDB ID's 1NFI. On the other hand for protein Hepatitis BX with PDB ID's 3I7H grid dimension were 126, 126 and 126 for X, Y and Z axes respectively. Gasteiger charges were assigned for all the compounds, and nonpolar hydrogen atoms were merged. All torsions of the ligand were allowed to rotate during docking. The value for the exhaustiveness of the search was 8. All graphic

manipulations and visualizations were performed using the AutoDock Tools and ligand docking with Autodock Vina [10].

## 3. Results and Discussion

### 3.1. GC-MS analysis

A total number of 6 phytocompounds have been identified in CCL (Fig. 1 and Table 1) among which Tetradecanoic acid (Myristic acid), Hexadecanoic acid, Linolenic acid, methyl ester, squalene, Heptacosane and stigmasterol are the main bioactive compounds observed.

### 3.2. Molecular Docking and hepatoprotective activity

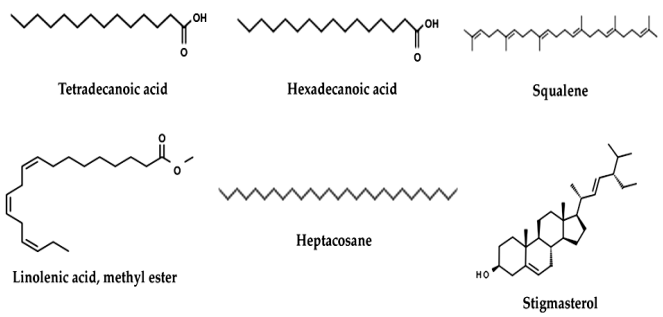


**Figure 1:** The structure of the phytocompounds present in *C. colebrookianum* leaf extract.

**Table 1.** List of phytocompounds identified in C. colebrookianum leaf extract by GC-MS analysis.

| Identified Compounds | Formula | Mol. Wt. † | RT |
|---|---|---|---|
| Tetradecanoic acid (Myristic acid) | $C_{14}H_{28}O_2$ | 227 | 28.84 |
| Hexadecanoic acid | $C_{16}H_{32}O_2$ | 255 | 31.71 |
| Linolenic acid, methyl ester | $C_{19}H_{32}O_2$ | 291 | 33.66 |
| Squalene | $C_{30}H_{50}$ | 409 | 45.16 |
| Heptacosane | $C_{27}H_{56}$ | 379 | 46.07 |
| Stigmasterol | $C_{29}H_{48}O$ | 411 | 52.81 |

The bioactive compounds of 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 ligands required to conduct the experiment are the compounds identified by GC-MS analysis of the plant extract. Upon a series of receptor-ligand interaction study, it was identified that each of the ligands has different binding affinity with the selected proteins. 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 (-8.7 kcal/mol) (Fig. 2 and Table 2). NFκβ protein and stigmasterol also has good binding affinity (-7.9 kcal/mol) and as seen in the secondary structure views (Fig. 3 and Table 2).

Hepatitis BX may act as the precursor for Hepatocellular carcinoma (HCC). Hepatitis BX promotes the expression of insulin-like growth factor

**Table 2:** Binding affinity of receptors (protein) with ligands (phytochemicals).

| Ligands | Binding Affinities (kcal/mol) | |
|---|---|---|
| | Hepatitis BX (3I7H) | NF-κβ (1NFI) |
| Tetradecanoic acid (Myristic acid) | -5.7 | -5.4 |
| Hexadecanoic acid | -6.4 | -5.4 |
| Linolenic acid, methyl ester | -4.9 | -3.8 |
| Squalene | -6.3 | -4.9 |
| Heptacosane | -5.2 | -3.8 |
| Stigmasterol | -8.7 | -7.9 |

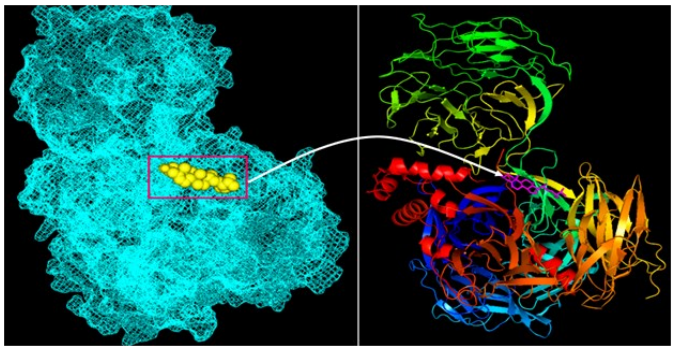

**Figure 2:** Molecular docking interactions of Stigmasterol with Hepatitis BX (3I7H).

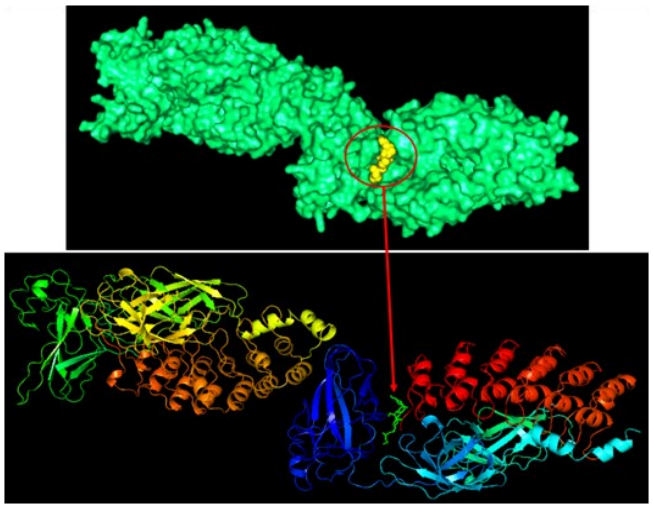

**Figure 3:** Molecular surface view of NF-κβ (1NFI) protein with Stigmasterol.

(IGF) in HCC. Thus blocking this protein with this phytochemical can reduce the chances of development of HCC in case of liver diseases. Proteins like NF-κB also showed good interactions with these phytochemicals. NF-κB controls cytokine production and cell survival, but in certain cases its regulation is related to cancer, inflammation and autoimmune diseases. Phytochemicals from the CCL extract act as suitable ligand for all these receptors. So, whether it is because of the individual bioactive phytochemical or the result of synergestic effects of all the biochemicals, *C. colebrookianum* can be considered to have medicinal benefits against hepatotoxicity.

## 4. Conclusions

In molecular docking study, we also studied the potential interactions and binding affinities of different compounds found in CCL with proteins from the human liver and discovered that a number of these compounds bind to different proteins quite strongly. Therefore, the current discovery may open a new avenue for understanding the causes of various diseases and disorders, which will aid in the development of new drugs.

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