Anti-Inflammatory activity and *Insilco* Approaches on root extract of *Decalepis hamiltonii* Wight & Arn

ABSTRACT

Aims: The present study was aimed to evaluate anti-inflammatory and antioxidant potential of methanol root extract of *Decalepis hamiltonii*. **Results** The methanolic extract showed *in vitro* anti-inflammatory activity inhibition cells 96.1%µg/ml. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was significant inhibited by the methanol (96.2) and inhibition addition the Nitric oxide production 25µg/ml 95% inhibition. The methanol extract of *Decalepis hamiltonii* shows a Thus, a very strong approach to In-silico docking and in vitro anti-inflammatory study for whole with these compounds/extract. **Conclusion.** From the study it is concluded that the root extract of *Decalepis hamiltonii* showing a strong approach to *In-silico* docking and anti inflammatory activity.

Keywords: *Decalepis hamiltonii;* .MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); Nitric oxide; Ferric reducing antioxidant power

1. Introduction

Medicinal plants have been used as age long remedies for human diseases because they contain components of therapeutic value. The plants are naturally large source of antioxidants, anticancer, antidiabetic agents they might be serve as leads for the development of novel drugs. The research and analysis on plants employed as pain-relievers and anti-inflammatory agents in traditional ethno medicine is one of the productive and logical strategies in the search for new drugs [1-2]. Free radicals, which have one or more un-paired electrons (superoxide, hydroxyl, peroxyl) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells [3-

6]. The increase in the prevalence of multiple drug resistance has shown down the development of new synthetic anti-inflammatory drugs and the new drug is necessary to search for new anti-inflammatory from alterative sources. Phytochemicals from medicinal plants showing anti-inflammatory activities have the potential of filling this need because of the structures of phytochemical different from those of the more studied and they're those of the more action may too very likely differ [7]. In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities [8-11] Inflammation are pathological condition in which highly reactive species are produced. Nitric oxide (NO) is a small diffusible molecule responsible for vasodilatation, neurotransmission and inflammation. This molecule is produced by the organism at a basal concentration. Nevertheless, under stimulation by pathogens, NO is generated in higher amounts by the inducible nitric oxide synthase (iNOS) in activated macrophages. The over production of NO is involved in the pathogenesis of septic shock, tissue damage, multiple organ dysfunctions and carcinogenesis processes [12]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide has been re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost. This *Decalepis hamiltonii* (Wight& Arn), popularly known as swallow root in English, belongs to the family *Asclepiadaceae.* It is a climber with stout, smooth branches shrub and a native of the forests of Deccan peninsula and Western Ghats of India. The leaves are curvaceous, orbicular or elliptical, with rounded tip. Its tubers are consumed as the pickles and the juice for its alleged health promoting properties The

Root has a sweet sarsaparilla-like taste; contains 9.2% fleshy matter and 8% woody core [13]. In this context the present research work is on antioxidant and anti-inflammatory activity of Decalepis hamiltonii (Wight & Arn).

2. Material and Methods

2.1 Collection of material

The Root of *Decalepis hamiltonii* has been collected from kolli hills, Namakkal district of Tamilnadu, India. The taxonomic identification of plant was done with comparing the Flora of Presidency of Madras, by Gamble J.S., 1921.

2.2 Preparation of solvent extraction

50gm of *Decalepis hamiltonii* root was packed in Soxhlet apparatus for extraction and 500 ml of methanol was used as solvent. Soxhlet was kept running for 72 hours, until the solvent color appears in the collection tube. Methanol was removed by evaporation using rotary vapour at not more than 40°C. The residue was then placed in an oven at 40°C for about 48hours to remove the moisture. The resulting dried mass was then powdered and used for further studies.

2.3 Ferric Reducing Antioxidant Power

The Ferric reducing antioxidant power of different parts of the various crude extracts of *Decalepis hamiltonii* was evaluated [14]. The samples were mixed with 2.5 ml of 0.2 M Phosphate buffer (pH 6.6) and 2.5 ml of 1 per cent potassium ferric cyanide. After the mixture was incubated at 50 ºC for 20 min, 2.5 ml of 10 per cent Trichloroacetic acid, 2.5 ml distilled water and 0.5 ml of 0.1 per cent ferric chloride was added and then the absorbance was measured at 700 nm against a blank. The blank consist of all the reagents without the test sample. The reducing power of Gallic acid was also

69 determined for a comparison. High absorbance of the reaction mixture indicates strong ferric reducing
70 antioxidant power.

71 **2.4 MTT assay**

72 The Peripheral blood mononuclear layer cell culture was trypsinized and the cell count
73 was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each
74 well of the 96 well micro titreplate, 0.1 mL of the diluted cell suspension (approximately 10,000
75 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was
76 flicked off, washed the monolayer once and 100 µL of different drug concentrations was added
77 to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO2
78 atmosphere and microscopic examination was carried out and observations recorded every 24
79 hours. After 72 hours, the drug solutions in the wells were discarded and 50 µL of MTT
80 (2mg/mL) prepared in Hank's balanced salt solution (HBSS) without phenol red was added to
81 each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO2
82 atmosphere. The supernatant was removed and 50 µL of propanol was added and the plates were
83 gently shaken to solubilize the formed formazan[15]. The absorbance was measured using a
84 microplate reader at a wavelength of 540 nm.

85 **2.5 Nitric Oxide Production**

86 The Nitric oxide (NO) production in cell culture were determined by measuring the
87 nitrite (NO_2) formation by using Griess assay [16]. Briefly, 100µL of griess reagent (Sigma-
88 Aldrich, USA) containing 1% sulfanilamide and 0.1% naphtylethyene diamine dihydrochloride
89 in 2.5% H_3PO_4 was added to 100 µl of cell culture supernatant and incubate for 15 minutes at
90 room temperature in the dark. The color density was measured at 540 nm using a microplate

reader (Molecular Devices, USA). A standard curve was generated in the same method using $NaNO_2$.

The compounds namely 2-Hydroxy-4-methoxybenzaldehyde was identified by GC-MS analysis were screened against the anti diabetic, anti - inflammatory and cancer protein. The compound details were retrieved from the Pubchem database and the chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chemsketch Software (www.acdlabs.com).

2.7 Protein Data Bank

The Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids. The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, WWPDB.

2.8 Preparation of protein structure

The structural information of the macromolecules determined by x-ray crystallographic and NMR methods are available in the PDB. The 3D structure protein Receptor (PDB I.D: 4IFI) was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) the water molecules were removed from protein file 4IFI before docking. Energy minimization by applying for CHARMM (Chemistry at Harvard Macromolecular Mechanics) force fields, It's a

program for macromolecular dynamics; it can be used for energy minimization, normal modes and crystal optimizations and also incorporates free energy methods for chemical and conformational free energy calculations.

2.9 PUBCHEM

The Pubchem bioassay data (http://pubchem.ncbi.nlm.nih.gov) is a public repository for biological Activities of small molecules and small interfering RNAs (siRNAs) hosted by the US National Institutes of Health (NIH). It archives experimental descriptions of assays and biological test results and makes the information freely accessible to the public. A Pubchem Bioassay data entry includes an assay description, a summary and detailed test results. Each assay record is linked to the molecular target, whenever possible, and is cross-referenced to other National Center for Biotechnology Information (NCBI) database records. 'Related Bioassays' are identified by examining the assay target relationship and activity profile of commonly tested compounds. A key goal of PubChem Bioassay is to make the biological activity information easily accessible through the NCBI information retrieval system- Entrez, and various web-based PubChem services. An integrated suite of data analysis tools is available to optimize the utility of the chemical structure and biological activity information within PubChem, enabling researchers to aggregate, compare and analyze biological test results contributed by multiple organizations. Describe the PubChem Bioassay database, including data model, bioassay deposition and utilities that PubChem provides for searching, downloading and analyzing the biological activity information.

2.10 Preparation of Ligand structures

The identified Chemical compound namely 2-Hydroxy-4-methoxybenzaldehyde was derived from *Decalepis hamiltonii* Wight & arn and this compound structure were retrieved from Pubchem online server both of these compounds were under investigation of Chemsketch (Chemically intelligent drawing interface free ware developed by Advance Chemistry Development, Inc., (http://www.acdlabs.com) was used to construct the structure of the ligands. The ligand molecules were generated and the three dimensional optimizations were done and then saved MOL file (a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule).

2.11 Drug likeliness prediction

Ligand property was predicted by using "Lipinski drug Filters" (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp). Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipinski filter helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures.

2.12 Docking analysis:

The docking analysis is performed by Argus lab 4.0.1 for the antidiabectic and cancer protein interacts with Gas-Chromatography of *Decalepis hamiltonii* 2-Hydroxy-4-methoxybenzaldehyde compound. The compound or ligand selected for based on Lipinski's rule of five. Fitting points are added to hydrogen bonding groups on the protein. The interaction between the binding pockets of target protein, antidiabetic protein and 3v6r inflammation protein and investigation compound to find out the accurate binding model for the active site of protein. The mechanism of ligand placement is based on binding site position. The protein ligand docking

energy values performance of this compound was based on the Scoring functions which is implemented in docking program to make various assumptions and implications to fit best complexes, which includes terms of hydrogen bonds employed by Argus lab 4.0.1 to rank the docked bases and to assess the binding site and the number of rotatable bonds present.

2.13 Ligand binding sites prediction

After docking the docked structure was saved as ".Pdb" file and further explored to predict the binding sites using "ligand explorer" software. The predicted binding sites, based on the binding energy, and amino acids make up the binding cavity. Here ligand binding site represents the site where the ligands most efficiently bind with the protein, among all the active site.

2.13 Discovery Studio Visualizer

The docking results were visualized using Accelyrs Discovery Studio 4.1 Visualizer. The discovery studio visualizer is also a free viewer that is designed to offer an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, script and other data. DS Visualizer is handier for analyzing the docking results.

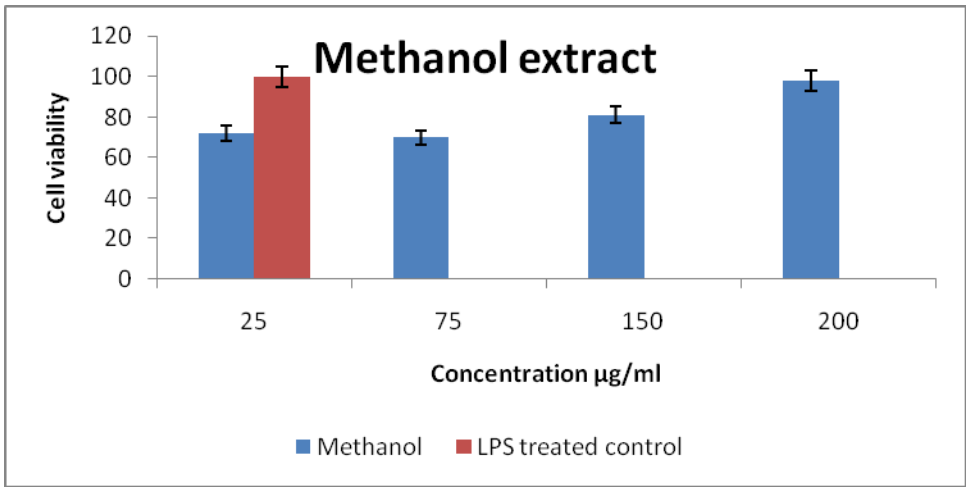
3.0 Results and Discussion

3.1 In-vitro Cytotoxicity activity

Cytotoxicity effect of the root extract of *Decalepis hamiltonii* in Peripheral Blood Mononuclear cells (PBMC)

MTT (3-(4,5 dimethylthiazole- 2yl) - 2, 5-Diphenyl Tetrazolium Bromide) assay is widely used in the *in vitro* evaluation of the biosafety of plant extracts. Determination of

cytotoxic effect of plant extracts is needed before studying the mechanism of action in various cells / cell lines. The cytotoxicity activity of methanolic root extract of *Decalepis hamiltonii* on cell viability was determined using cell line culture at various concentrations is shown in Fig 1. The values of cell viability were found to be in the range of 68% to 98% at various concentrations tested. The highest concentration of 200µg/ml showed moderate Cytoxicity. Cell viability was evaluated by MTT assay, hence the concentrations of sample (cell viability ≥90) were selected for subsequent iNOS inhibition experiment.

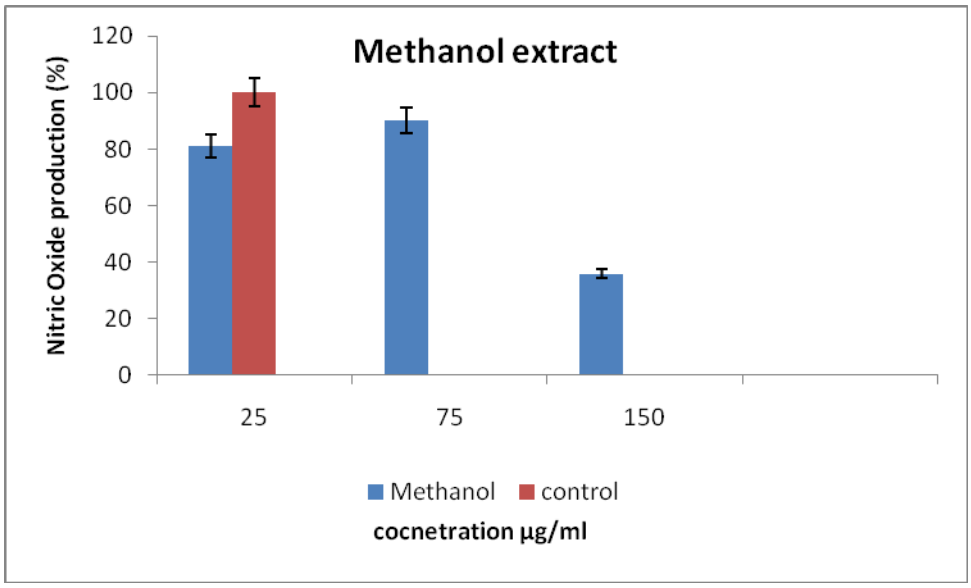


Results are expressed as mean ± Standard deviation

Figure. 1 Cytotoxicity effect of the root extract of *Decalepis hamiltonii*

3.2 Inhibition of Lipopolysaccharide – induced iNOS production by root extracts

To investigate the inhibitory effects of the methanolic extracts from the LPS–induced iNOS production in peripheral blood mononuclear cell (PBMC) the accumulation of iNOS in the culture medium is measured. As shown in Figure 2, root extracts significantly suppressed LPS-induced iNOS product in a dose-dependent manner. The root extract at 75µg/ml significantly inhibited the production of iNOS up to 90.2±0.86.



Results are expressed as means ± standard deviation

Fig. 2 Nitric oxide effect of the root extract of *Decalepis hamiltonii*

3.3 **Ferric reducing antioxidant power**

Ferric reducing antioxidant power of Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of root plant parts of *Decalepis hamiltonii* is shown in Fig. 3. IC_{50} values were calculated as the amount of antioxidant present in the sample necessary to decrease the initial ferric reducing antioxidant concentration by 50%. Petroleum ether, Chloroform, Ethyl acetate and Methanol root extracts of *Decalepis hamiltonii* exhibited the highest activity. The IC_{50} values of Methanol extracts of *Decalepis* root and Gallic acid values were 392.7, 442.5, 311.4 and 171.02 µg/ml respectively.

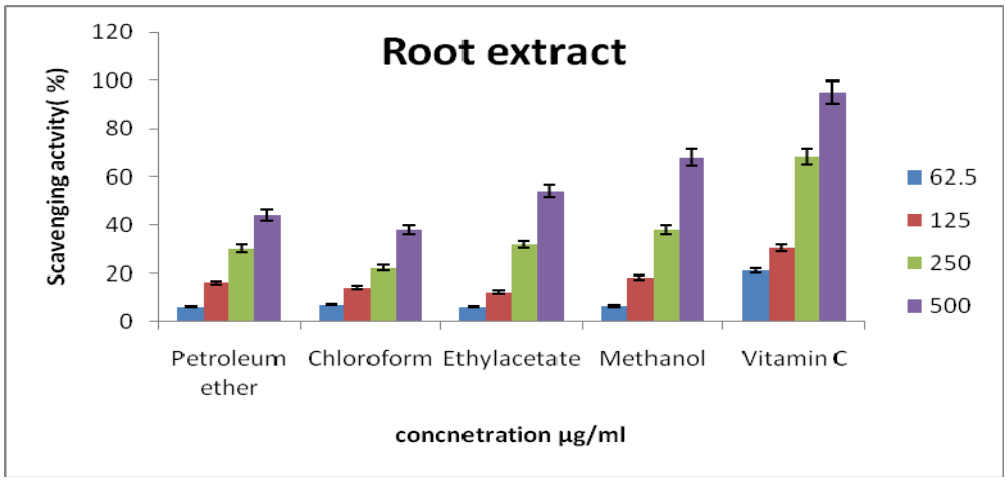


Fig. 3 In silico* analysis of anti- inflammatory activity of 2-hydroxy-4-methoxybenzaldehyde from root extract of *Decalepis hamiltonii

The molecular interaction between the ligand and the protein (receptor) was investigated using the Ligand fit protocol. The 2-Hydroxy- 4 -Methoxy Benzaldehyde was docked with active site inflammation receptor protein 3V6R-docking energy-17.45 kcal/mol. 3D structure and 2D structure interaction to interact with Targeted protein; amino acid region and distance. The docking studies further confirmed the anti-diabetic property of the bioactive compounds via GC-MS analysis and suggested that first time demonstrated a strong 3v6r (Inflammation) inhibitory property of this extract, maybe due to the identified bioactive compounds (Figure.4).

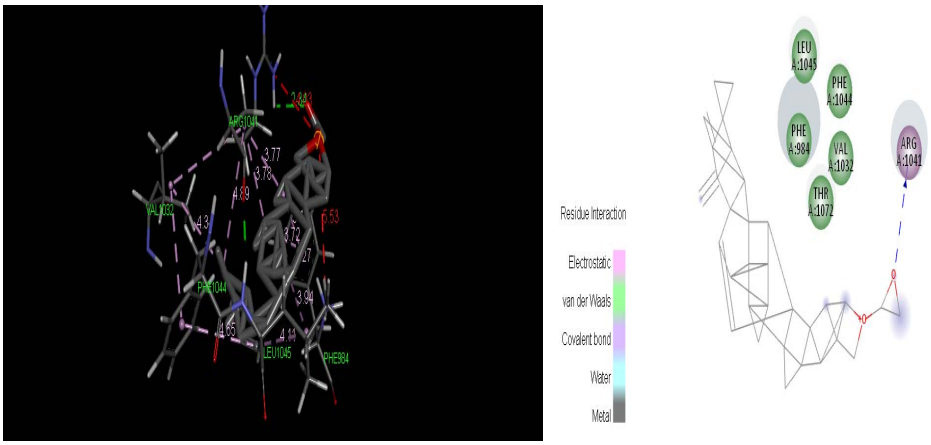


Figure.4 3D and 2D structures for amino acid regions for 2-Hydroxy-4- Methoxy Benzaldehyde interact with Targeted protein

In the present study methanolic root extract of *Decalepis hamiltonii* was found to have maximum inhibition concentration (99.5% at 200 µg/ml. Similar results were observed [17], who had shown moderate cytotoxicity of *Polygonum chinensis* extracts (400- 125 µg/ml). [18] also compared root, stem and leaf extracts of *Jatropha curcas* in anti-inflammatory activity study towards carrageen an induced *rat paw edema*. Groups of albino rats were injected by inflammation inducer (Carrageenan) and the levels of inflammation mediators (histamine, serotonin and kinin) were monitored. Their results showed that root extract inhibited histamine, serotonin and kinin released up to 70% - 80% compared to leaf (50% - 55%) and stem (40% - 45%). However, these studies did not indicate the nature of compounds involved in the anti-inflammatory activity.

Ferric reducing properties are generally depend on the presence of reductions which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom [19].The present study of the ferric radical activity of the all the extracts of the *Decalepis hamiltonii* leaves, stem and root were analyzed. The root extract was found to increase in a concentration depended manner. Among all the four extracts, methanol root extract was recorded the highest scavenging activity of 78.0% at a concentration of 500µg/ml. The results suggest that methanol extracts have more reducing power than ethyl acetate, petroleum ether and chloroform but not as efficient as standard gallic acid. Similar type of work has also been carried out in *Azima tetracantha* [20]. It is believed that antioxidant activity and reducing power are related [21]. Reuctones donate a hydrogen atom and inhibit LPO by donating hydrogen. Atom and there by terminating the free radical chain reaction [22].

3.5 Conclusion

Thus, a very strong approach to *In-silico* docking and in vitro anti-inflammatory study for whole with these compounds/extracts. Further studies regarding isolation and characterization of bioactive principles responsible for this action may be further carried out in future.

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