

1 **Anti-Inflammatory activity and *Insilco* Approaches on root extract of *Decalepis hamiltonii***

2 **Wight & Arn**

3 **ABSTRACT**

4 **Aims:** The present study was aimed to evaluate anti-inflammatory and antioxidant  
5 potential of methanol root extract of *Decalepis hamiltonii*. **Results** The methanolic extract  
6 showed invitro anti-inflammatory activity inhibition cells 96.1%µg/ml respectively. MTT assay  
7 was significant inhibited by the methanol (96.2) and inhibition addition the NO production  
8 25µg/ml 95% inhibition. The methanol extract of *Decalepis hamiltonii* shows a Thus, a very  
9 strong approach to In-silico docking and in vitro anti-inflammatory study for whole with these  
10 compounds/extracts. **Conclusion.** From the study it is concluded that the root extract of  
11 *Decalepis hamiltonii* showing a strong approach to InSilico docking and anti inflammatory  
12 activity.

13

14 **Keywords:** *Decalepis hamiltonii*; MTT assay; NO; FRAP

15 **1. Introduction**

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

**Comment [A1]:** Where are the two value?

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**Comment [A3]:** Mention the full name

**Comment [A4]:** Mention the full name

24 6]. The increase in the prevalence of multiple drug resistance has shown down the development  
25 of new synthetic anti-inflammatory drugs and the new drug is necessary to search for new anti-  
26 inflammatory from alternative sources. Phytochemicals from medicinal plants showing anti-  
27 inflammatory activities have the potential of filling this need because of the structures of  
28 phytochemical different from those of the more studied and they're those of the more action may  
29 too very likely differ [7]. In this growing interest, many of the Phytochemical bioactive  
30 compounds from a medicinal plants have shown many pharmacological activities [8-11]  
31 Inflammation are a pathological condition in which highly reactive species are produced. Nitric  
32 oxide (NO) is a small diffusible molecule responsible for vasodilatation, neurotransmission and  
33 inflammation. This molecule is produced by the organism at a basal concentration. Nevertheless,  
34 under stimulation by pathogens, NO is generated in higher amounts by the inducible nitric oxide  
35 synthase (iNOS) in activated macrophages. The over production of NO is involved in the  
36 pathogenesis of septic shock, tissue damage, multiple organ dysfunctions and carcinogenesis  
37 processes [12]. Natural products have contributed significantly towards the development of  
38 modern medicine. Recently traditional medicine worldwide has been re-evaluated by extensive  
39 research on different plant species and their active therapeutic principles. The rich wealth of  
40 plant kingdom can represent a novel source of newer compounds with significant anti-  
41 inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy,  
42 low incidence of serious adverse effects, and low cost. This *Decalepis hamiltonii* (Wight & Arn),  
43 popularly known as swallow root in English, belongs to the family *Asclepiadaceae*. It is a  
44 climber with stout, smooth branches shrub and a native of the forests of Deccan peninsula and  
45 Western Ghats of India. The leaves are curvaceous, orbicular or elliptical, with rounded tip. Its  
46 tubers are consumed as the pickles and the juice for its alleged health promoting properties The

**Comment [A5]:** Picture of the plant is more descriptive

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

## 50 **2. Material and Methods**

### 51 **2.1 Collection of material**

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

### 55 **2.2 Preparation of solvent extraction**

56 50gm of *Decalepis hamiltonii* root was packed in Soxhlet apparatus for extraction and  
57 500 ml of methanol was used as solvent. Soxhlet was kept running for 72 hours, until the solvent  
58 colour appears in the collection tube. Methanol was removed by evaporation using rotavapour at  
59 not more than 40°C. The residue was then placed in an oven at 40°C for about 48hours to  
60 remove the moisture. The resulting dried mass was then powdered and used for further studies.

### 61 **2.3 Ferric Reducing antioxidant Power**

62 The Ferric reducing antioxidant power of different parts of the various crude extracts of  
63 *Decalepis hamiltonii* was evaluated by [14] The samples were mixed with 2.5 ml of 0.2 M Phosphate  
64 buffer (pH 6.6) and 2.5 ml of 1 per cent potassium ferric cyanide. After the mixture was incubated at 50  
65 °C for 20 min, 2.5 ml of 10 per cent TCA, 2.5 ml distilled water and 0.5 ml of 0.1 per cent ferric  
66 chloride was added and then the absorbance was measured at 700 nm against a blank. The blank  
67 consist of all the reagents without the test sample. The reducing power of gallic acid was also

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**Comment [A7]:** What is this refer to?

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

#### 70 **2.4 MTT assay**

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

#### 84 **2.5 Nitric Oxide Production**

85 The Nitric oxide (NO) production in cell culture were determined by measuring the  
86 nitrite (NO<sub>2</sub>) formation by using Griess assay as described by [16]. Briefly, 100 $\mu$ L of griess  
87 reagent (Sigma-Aldrich, USA) containing 1% sulfanilamide and 0.1%  
88 naphthylethyenediaminedihydrochloride in 2.5% H<sub>3</sub>PO<sub>4</sub> was added to 100  $\mu$ L of cell culture  
89 supernatant and incubate for 15 minutes at room temperature in the dark. The color density was

**Comment [A8]:** From any tissue

**Comment [A9]:** Put 5 in correct position ?

**Comment [A10]:** Put degree centigrade in its position

**Comment [A11]:** Must add the name of the author

90 measured at 540 nm using a microplate reader (Molecular Devices, USA). A standard curve was  
91 generated in the same method using NaNO<sub>2</sub>.

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

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**Comment [A13]:** What is this refer to

**Comment [A14]:** Spelling mistake

## 97 2.7 Protein Data Bank

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

**Comment [A15]:** What is this refer to?

## 106 2.8 Preparation of protein structure

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

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

## 115 **2.9 PUBCHEM**

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

## 132 **2.10 Preparation of Ligand structures**

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

#### 141 **2.11 Drug likeliness prediction**

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

#### 147 **2.12 Docking analysis:**

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

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**Comment [A18]:** What is this refer to?

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

### 159 **2.13 Ligand binding sites prediction**

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

### 165 **2.13 Discovery Studio Visualizer**

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

## 170 **3.0 Results and Discussion**

### 171 **3.1 In-vitro Cytotoxicity activity**

#### 172 **Cytotoxicity effect of the root extract of *Decalepis hamiltonii* in Peripheral Blood**

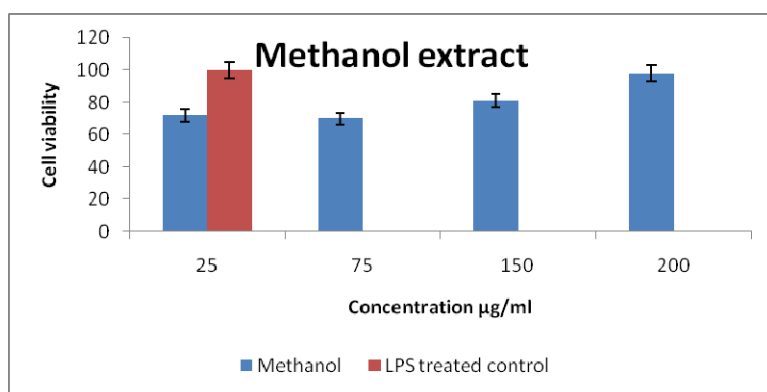
##### 173 **Mononuclear cells (PBMC)**

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

**Comment [A19]:** What is this refer to?



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



183

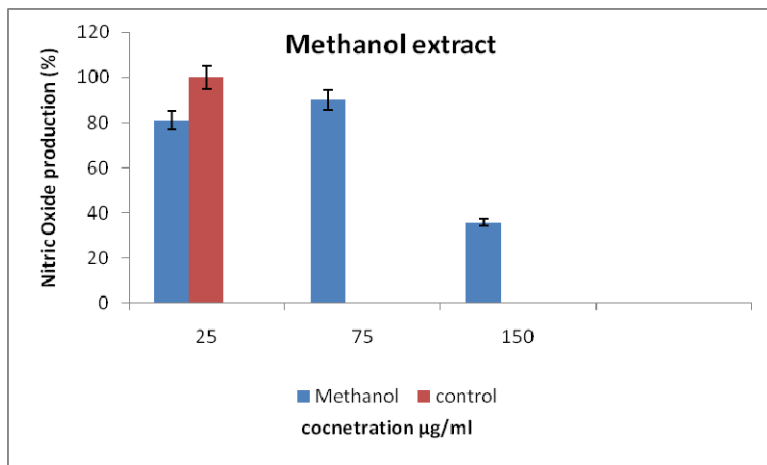
184 Results are expressed as mean  $\pm$  Standard deviation

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

### 186 3.2 Inhibition of LPS – induced iNOS production by root extracts

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

Comment [A20]: Mention the full name



192

193 Results are expressed as means  $\pm$  standard deviation

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

**Comment [A21]:** Why you do not use 200 concentration?

195 **3.3 Ferric reducing antioxidant power**

196 Ferric reducing antioxidant power of Petroleum ether, Chloroform, Ethyl acetate and

197 Methanol extracts of root plant parts of *Decalepis hamiltonii* is shown in Fig. 3.  $IC_{50}$  values were

**Comment [A22]:** Mention the full name

198 calculated as the amount of antioxidant present in the sample necessary to decrease the initial

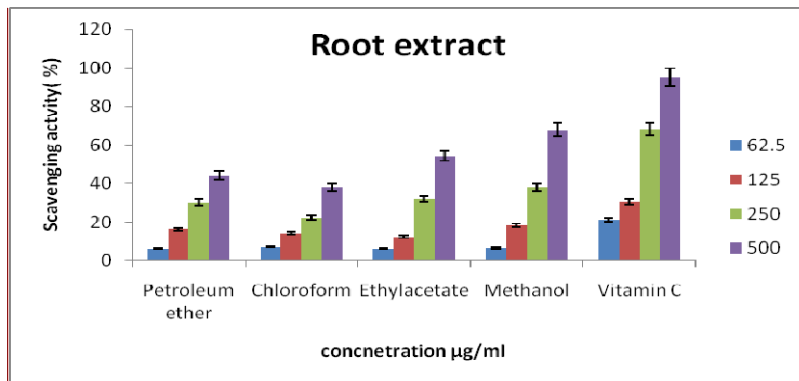
199 ferric reducing antioxidant concentration by 50 percentage. Petroleum ether, Chloroform, Ethyl

200 acetate and Methanol root extracts of *Decalepis hamiltonii* exhibited the highest activity. The

201  $IC_{50}$  values of Methanol extracts of *Decalepis* root and Gallic acid values were 392.7, 442.5,

**Comment [A23]:** Two compare group in front of four values

202 311.4 and 171.02  $\mu\text{g/ml}$  respectively.

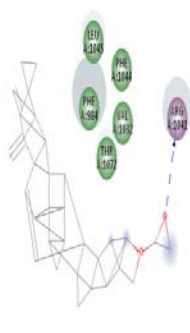
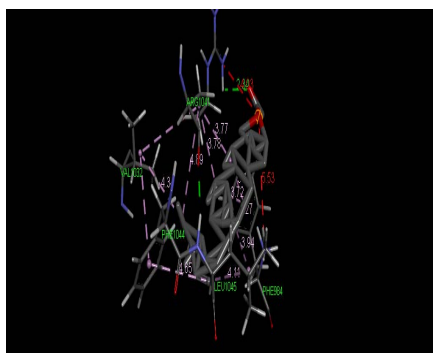


Comment [A24]: What are these values refer to?

203

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

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



213

214 **Figure.4 3D and 2D structures for amino acid regions for 2-Hydroxy-4- Methoxy**

215 **Benzaldehyde interact with Targeted protein**

216 In the present study methanolic root extract of *Decalepis hamiltonii* was found to have maximum  
217 inhibition concentration (99.5% at 200 µg/ml. Similar results were observed [17], who had  
218 shown moderate cytotoxicity of *Polygonum chinensis* extracts (400- 125 µg/ml). [18] also  
219 compared root, stem and leaf extracts of *Jatropha curcas* in anti-inflammatory activity study  
220 towards carrageen an induced *rat paw edema*. Groups of albino rats were injected by  
221 inflammation inducer (Carrageenan) and the levels of inflammation mediators (histamine,  
222 serotonin and kinin) were monitored. Their results showed that root extract inhibited histamine,  
223 serotonin and kinin released up to 70% - 80% compared to leaf (50% - 55%) and stem (40% -  
224 45%). However, these studies did not indicate the nature of compounds involved in the anti-  
225 inflammatory activity.

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

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Comment [A27]: What is this?

Comment [A28]: What is this?

Comment [A29]: What is this?

237 **3.5 Conclusion**

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

242 **Reference**

**Comment [A30]:** Not uniformly standardized

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