ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of *Invitro* Antioxidant and *Invivo* Hepatoprotective Potency of *Myristica Malabarica*

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ABSTRACT

Objective of the present study was to evaluate methanolic extract of *Myristica malabarica* for its hepatoprotective potential. Antioxidant activity of the extract was evaluated by using Diphenyl picryl hydrazyl (DPPH) radical scavenging, Hydrogen peroxide scavenging, 2, 2'-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging and Nitric oxide (NO) radical scavenging activity followed by determination of Total Phenol content. Hepatoprotective activity of the extract was evaluated by carbon tetrachloride (CCl_4) induced liver damage model in rats. Molecular mechanism of the hepatoprotective activity was studied through insilico approach using molecular docking against Nuclear Factor *kappa* B (NFkB) and Pregnane X receptors to identify the possible leads responsible for claimed activity. The extract demonstrated a significant dose dependent antioxidant activity with IC_{50} values at 0.02 mg/ml in DPPH assay, 0.107 mg/ml in Scavenging of hydrogen peroxide, 1.6 µg/ml in ABTS radical cation decolorization assay and 0.5 mg/ml in Nitric oxide scavenging assay which was comparable with that of Ascorbic acid. Animal groups treated with CCl_4 recorded significant rise in serum markers reflecting hepatic damage. Pretreatment of the rats with methanolic extract of *M. malabarica* (200mg/kg *p.o*) inhibited the increase in serum levels of total bilirubin, total protein, serum alanine transaminase, aspartate transaminase and alkaline phosphatase reflecting the liver protection by crude drug and the data were comparable with silymarin (100mg/kg po). The present studies indicates that *M. malabarica* stem bark have significant free radical scavenging, hepatoprotective activity and possibility of Prunetin and Biochanin A becoming lead candidates for liver protection.

Keywords: *Myristica malabarica*; Antioxidant; hepatoprotective; *Insilco* molecular docking.

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ISSN: 0975-8585

INTRODUCTION

The plant *M. malabarica* L., (Myristicaceae) is known for many medicinal properties such as indigestion, ulcers, wounds, aphrodiasic, as rejuvenator in bladder, inflammation, cough, diarrhea, dropsy, liver disorders, paralysis, rheumatism, urinary calculi, vomiting, bronchitis, fever, burning sensation, relief of pain in muscles, sprains and sores [1]. The plant contains many active constituents like 7, 4-dimethoxy-5 hydroxyl isoflavone, biochanin A, prunetin, 1,3-diarylpropanol and alpha-hydroxyldihydrochalcone [2], 2-acylresorcinol, diarylnonanoids, malabaricone C [3], malabaricone A [4], Malabaricones A-D, diarylnonanoids (Talukdar et al 2000). The plant is known for its antioxidant activity [5]. Ethno botanical survey in the Western Ghats of Uttarkannada district, Karnataka, India revealed that the stem bark of *M. malabarica* is used by the ethnic group to treat jaundice, wound healing and in skin diseases (personal communication). Though the plant is used by the ethnic group of Western Ghats for treating jaundice, no scientific study is carried out to evaluate its hepatoprotective potency. Hence, the present study was undertaken to evaluate the hepatoprotective potency of the *M. malabarica* through CCl_4 induced hepatic damage model and its ability to scavenge free radicals. This study was also supplemented with *Insilico* analysis where the phytoconstituents elicited from literature survey were docked with target receptors downloaded from Protein Data Bank to predict the preferred orientation and binding affinity of lead molecule.

MATERIALS AND METHODS

Collection and extraction of crude drug

Stem bark of *M. malabarica* were collected from the Sirsi range forest, Karnataka State, during June 2011. Taxonomic authenticity was confirmed by the corresponding author and voucher specimens are deposited in the departmental herbaria (BKM- 133, BKM-134) as authentic specimen for future reference. The stem bark was shade dried, powdered mechanically (Sieve No. 10/44) and stored in airtight containers. About 250g of the powdered material was subjected to soxhlation, it was first defatted with petroleum ether (Hi-Media, Bangalore) and then exhaustively extracted with methanol (Hi-Media, Bangalore) for 48 hrs. The solvent was distilled off at low temperature under reduced pressure using rotory flash evaporator (Buchi, Flawil, Switzerland.). The yield was 29.3 % w/w.

Screening of antioxidant activity

Determination of Total Phenol by Folin-Ciocalteu Assay

The total phenolic content of crude methanolic extract was determined by the Folin-Ciocalteu method [6]. 1 ml of extract of various concentrations was added to 5 ml of 1:10 diluted FC reagent followed by 4 ml of 1 M Sodium carbonate solution. After 30 minutes of incubation in dark at room temperature, the absorbance was measured at 750 nm using UV-visible spectrophotometer. A calibration curve was constructed using different concentrations of standard Gallic acid. All readings were performed in triplicates and the level of Total Phenol in the extract was calculated from the standard calibration curve. Results were expressed in gallic acid equivalents (mg GAE/g).

Evaluation of DPPH free radical scavenging activity

The free radical scavenging activity of methanolic extract was studied by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay [7]. 2 ml of DPPH (100 µM) was mixed with various dilution ranging from 200µg to 1000µg of extract. After 10 minutes of incubation in dark, the absorbance was measured at 517 nm. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1: $[(A_0\text{-}A)/A_0]$ X 100, where A_0 is the absorbance of reagent blank and A is the absorbance of the test sample. The concentrations of plant extract and Ascorbic acid standard was plotted in X-axis against respective percentage inhibition in Y-axis and their IC_{50} values were calculated by extrapolating the graph.

Evaluation of Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). 1 ml of methanolic extract of varying concentrations was added to 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm after 10 minutes at room temperature [8]. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1.

Evaluation of Antioxidant Activity ABTS radical cation decolorization assay

To generate ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid) radical cation, 50 ml of 2 mM ABTS and 0.3 mL of 17 mM potassium persulfate were mixed together and incubated in the dark for 12-16 h to develop prussian blue colored

$ABTS^{\cdot+}$ solution which has an absorption maxima at 734 nm [9]. To determine scavenging activity of extracts, 400 µl of methanolic extracts of different concentrations were added to 320 µl of $ABTS^{\cdot+}$ solution. The absorbance was measured at 734 nm after 10 minutes incubation at room temperature. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1. The percentage inhibition of plant extract, ascorbic acid standard was plotted against respective concentrations used and their IC_{50} value was calculated by extrapolating the graph.

Evaluation of Antioxidant Activity Nitric oxide scavenging assay

Nitric oxide radical scavenging was estimated on the basis of modified Griess Illosvoy reaction [10]. 6 ml reaction mixture containing 2 ml of 10 mM sodium nitroprusside, 1 ml phosphate buffered saline and 1 ml methanolic extracts of different concentration was incubated at 25° C for 150 min. After incubation, 1 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Further 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25° C in diffused light. The absorbance of pink colored chromophore formed was measured at 540 nm against the corresponding blank solutions. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1. The percentage inhibition of plant extract and ascorbic acid standard was plotted against respective concentrations used. IC_{50} value was calculated by extrapolating the graph.

Evaluation of Hepatoprotective Activity

Drug formulation

Oral suspensions containing 100mg/ml and 200mg/ml of the methanol stem bark extracts were prepared in 1 % w/v gum tragacanth.

Animals

Male Wistar albino rats, each weighing 150-200 g were procured from the National College of Pharmacy, Shimoga and were maintained at standard housing conditions. The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum during the experiment. The study was permitted by the Institutional Animal Ethical Committee with Reg No. 144/1999/CPCSEA/SMG.

Acute toxicity studies

The stem bark methanol extract was found to be non toxic up to the dose of 2000mg per kg b.w. In the present study 100mg/kg p.o. and 200 mg/kg p.o. dose was selected to assess the hepatoprotective activity of the plant [11].

Evaluation of hepatoprotective activity

The animals were divided into five groups of six rats each. The animals in group I served as control and received the vehicle (1ml/kg/day of 1 % w/v gum tragacanth p.o.) for 14 days. All the animals of group II to V received 0.1ml/kg/day of CCl_4 (Hi-Media, Bangalore) for 14 days. Group III animals received the standard drug Silymarin (Ranbaxy Lab. Dewas) in the dose of 100 mg/kg/day p.o. for 14 days. Methanol stem bark extracts of *M. malabarica* were administered to the animals of group IV and V in the dose of 100mg/kg/day p.o. and 200mg/kg/day p.o. respectively for 14 days. The CCl_4, Silymarin and the extracts were administered concomitantly to the respective groups of animals. The animals of all the groups were sacrificed on 14th day under light ether anesthesia. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and was subjected to biochemical investigation viz., total bilirubin [12], total protein [13], serum alanine transaminase, aspartate transaminase [14] and alkaline phosphatase [15]. Results of biochemical estimations were recorded as mean ± SE of six animal in each group. The data was subjected to one way ANOVA 0.001 was considered as statistically * followed by Dunnett`s test P values significant.

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Histopathology

The liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6h. They were processed for paraffin thickness using microtome embedding. The sections were taken at 5 µm processed in alcohol-xylene series and were stained with alum haematoxylin and eosin [16]. The sections were examined microscopically for the evaluation of histopathological changes.

***Insilico* study**

To study the molecular mechanism, active constituents such as biochanin A, prunetin, malabaricone C, malabaricone A, malabaricones B and malabaricones D were selected. Chem Sketch of ACDLABS 10.00 software was used to design the ligands followed by 3D optimization. The Sybyl Mol2 format files of these ligands were converted into Protein data bank (pdb) format using Open Babel software [17]. The potent target receptors involved in hepatoprotection namely NFkB and Pregnane X receptor were elicited from literature survey [18, 19,20] and their respective pdb files ID: 1VKX [21] and ID: 1ILG [22] were retrived from Protein data bank. Gastieger atom charges, solvent deletion and hydrogens were added into the receptors files for the preparation of receptor in docking simulation by UCSF Chimera [23]. Docking analysis was done using Autodock 4.2 [24]. Torsions in active constituents were set to 6 and non-polar hydrogens present in receptors were merged. Files were saved in .pdbqt format. AutoGrid 4.2 program, supplied with AutoDock 4.2 was used to produce grid maps. The grid box size was set at 62, 62 and 62 A° (x, y, and z) to include all the amino acid residues present in the active site of macromolecules. The Lamarckian Genetic Algorithm (LGA) was chosen for the search of best conformers with lowest binding energy. Results were analyzed to study the interactions, binding energy, hydrogen bond interactions and the binding distance between the hydrogen bond donors and acceptors for the best conformers.

RESULTS

Antioxidant Activity

In the present investigation, it was found that methanolic plant extract has total phenolic content 0.1 mg/ml and is equivalent to 0.0825 mg/ml gallic acid exerting antioxidant effect as free radical scavengers. Methanolic extract exhibited good IC_{50} values (Table 1) at 0.02 mg/ml in DPPH free radical scavenging assay (Figure 1.1), 0.107 mg/ml in scavenging of hydrogen peroxide assay(Figure 1.2), 1.6 µg/ml in ABTS radical cation decolorization assay (Figure 1.3) and 0.5 mg/ml mg/ml in Nitric oxide scavenging assay (Figure 1.4) respectively. The extract demonstrated scavenging of free radicals in a concentration dependent manner and was comparable with standard Ascorbic acid.

Hepatoprotecive Activity

Effect of ethanol stem bark extract of *M. malabarica* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in table 2. At the end of 14 days treatment, blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase but the total protein level decreased reflecting the liver injury caused by CCl_4. Whereas blood samples from the animals treated with methanol stem bark extract of *M. malabarica* showed significant decrease in the levels of serum markers and significant increase in total protein indicating the recovery of hepatic cells. Among the two doses of the extract tested significant protection against CCl_4 induced hepatic damage was evident in the animal groups treated with 200 mg/kg p.o. Histological profile of control animal showed normal hepatocytes (Figure 2.1), the section of liver of the group II animals exhibited severe intense centrilobular necrosis (N), vacuolisation (Figure 2.2). The liver tissue sections of silymarin treated animals showed normal hepatic architecture (Figure 2.3). The liver tissue sections of the animals treated with methanol (Figure 2.4) at the dose of 100 mg/kg p.o. and 200 mg/kg p.o. exhibited significant liver protection against CCl_4 induced liver damage as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration.

Insilco* studies on mechanism of hepatoprotection by phytoconstituents of *M. malabarica

Present docking studies of the lead molecules revealed, important interactions operating at the molecular level like hydrogen bond interactions hydrogen bond distance between the donor and acceptor atoms (table 3 and table 4). All the active constituents of *M. malabarica* exhibited considerably low binding energy. Among the leads, Malbaricone D was found to be the best target drug for NFkB receptor as it exhibited lowest binding energy. Similarly Biochanin A for Pregnane X receptor. Malbaricone D cluster rank 1 with lowest binding energy -6.49 kcal/mol had 3 hydrogen bond interactions at residues LYS476,

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ARG540 and LYS507 with cluster reference RMSD 66.51. Hydrogen bond distance between the donor and acceptor atoms was found to be 2.064, 1.851 and 1.98 respectively. Docking interaction between the ligand and the macromolecule is shown in figure 3. Biochanin A cluster rank 1 with lowest binding energy -7.87 kcal/mol had 1 hydrogen bond interaction at residue GLN124 with cluster reference RMSD 65.99. Hydrogen bond distance between the donor and acceptor atoms was found to be 1.999. Docking interaction between Biochanin A and the Pregnane X receptor is shown in figure 4.

DISCUSSION

Carbon tetrachloride has been widely used for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsome [25]. The CCl_4-intoxication is characterized by a hepatic syndrome of cytolysis [26]. The toxicity of CCl_4 depends on its reductive metabolization which generates reactive free radicals leading to hepatocellular necrosis. The reactive free radical metabolites of CCl_4 produce lipidic peroxidation of biomembranes, enzymatic inhibition and covalently bind to the cellular macromolecules. The disturbance of cellular calcium homeostasis related to the lipidic peroxidation of the biomembranes represents the irreversible stage of the process which leads to the necrosis of hepatocytes by karyolysis or by acidophilic necrosis [27].

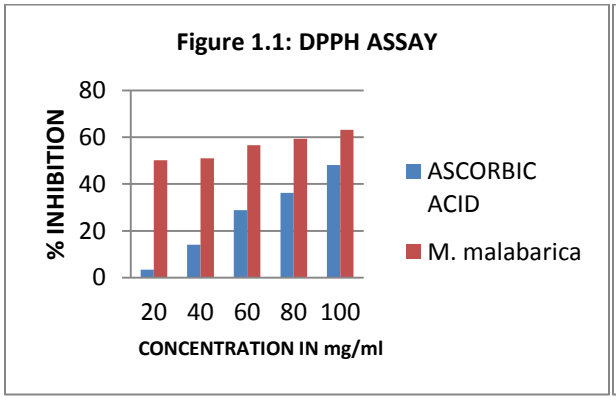

Figure 1.1: % Inhibition of methanol stem bark extract of *M. malabarica* and the standard Ascorbic acid in DPPH assay.

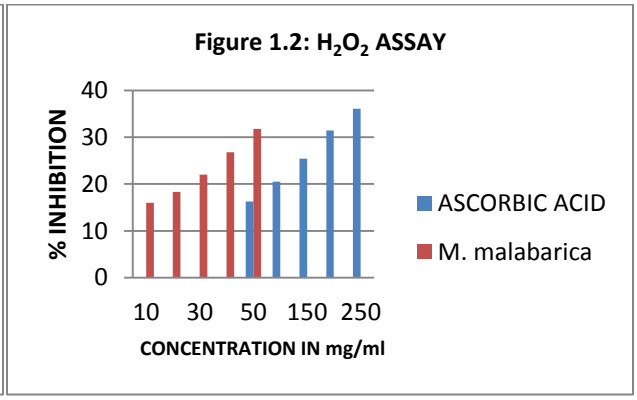

Figure 1.2: % Inhibition of methanol stem bark extract of *M. malabarica* and the standard Ascorbic acid in H_2O_2 assay.

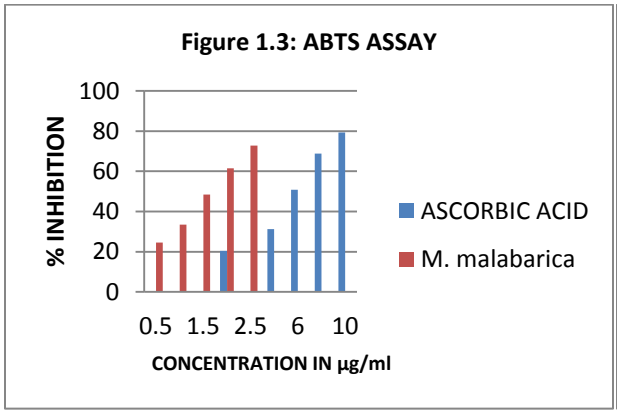

Figure 1.3: % Inhibition of methanol stem bark extract of and the standard Ascorbic acid in ABTS assay.

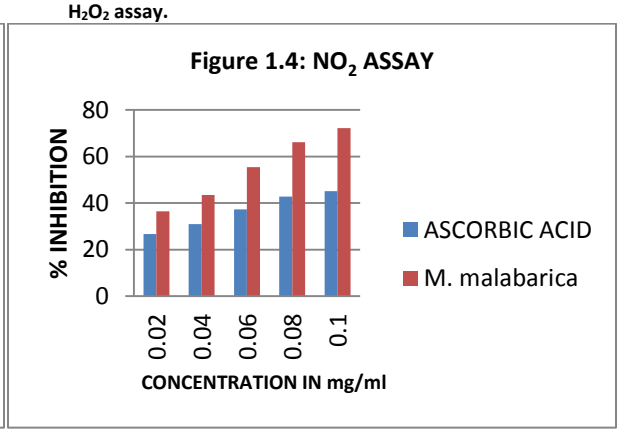

Figure 1.4: % Inhibition of methanol stem bark *M. malabarica* extract of *M. malabarica* and the standard Ascorbic acid in NO_2 assay.

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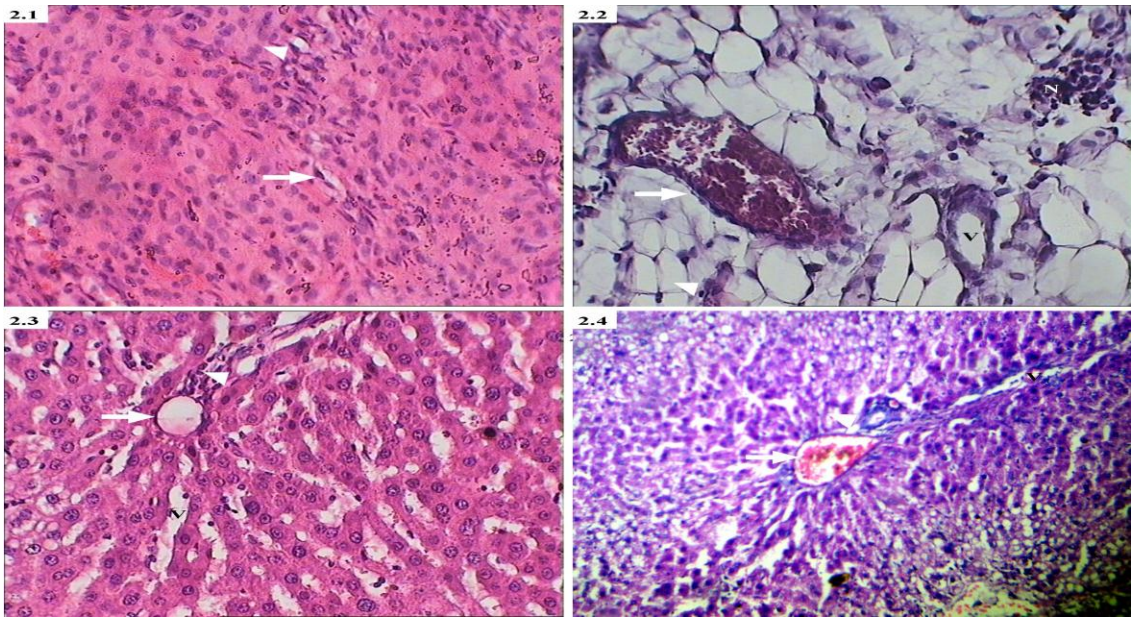

FIGURE LEGENDS:

Figure 2.1: Section of the liver tissue of control animal showing normal histology, hepatic artery (arrow) and bile duct (arrow head). (H & E, 100X)

Figure 2.2: Section of the liver tissue of animal treated with CCl_4 showing a central hepatic vein (V) and necrosis (N). (H & E, 100X)

Figure 2.3: Section of the liver tissue of silymarin treated animals showing normal hepatocytes with central hepatic vein (V), hepatic artery (arrow) and bile duct (arrow head). (H & E, 100X)

Figure 2.4: Section of the liver tissue of methanol stem bark extract of *Myristica malabarica* treated animals showing normal arrangement of hepatocytes around the portal vein (V), hepatic artery (arrow), bile duct (arrow head), absence of necrosis and fatty vacuoles. (H & E, 100X)

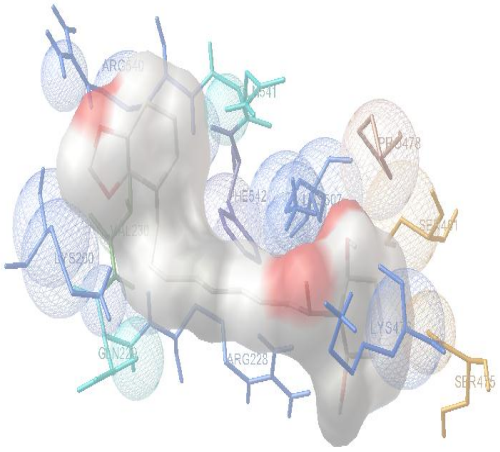

Figure 3: Docking interaction between the Malabaricone D and the NFkB receptor

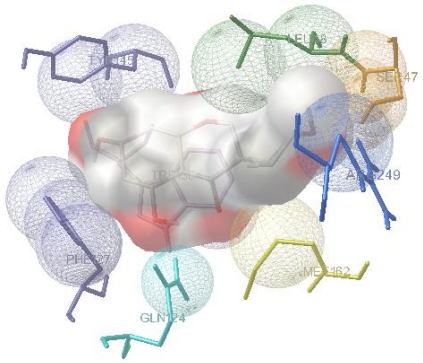

Figure 4: Docking interaction between the Biochanin A and the Pregnane X receptor

TABLE 1: Assays performed with IC_{50} values of methanol stem bark extract of *M. malabarica* and the standard Ascorbic acid

Sl No	ASSAY	IC_{50} Value of Ascorbic acid	IC_{50} Value of Plant Extract
1.	DPPH Free radical scavenging activity	0.12 mg/ml	**0.02 mg/ml**
2.	Scavenging of hydrogen peroxide	0.4 mg/ml	**0.107 mg/ml**
3.	ABTS radical cation decolorization assay	5.8 µg/ml	**1.6 µg/ml**
4.	Nitric oxide scavenging activity	1.1 mg/ml	**0.5 mg/ml**

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TABLE 2: Effect of methanol stem bark extract of *M. malabarica* on CCl_4 induced hepatotoxicity in rats.

.Group (N)	Total Bilirubin (mg/dl)	Total Protein (gm %)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (1% gum tragacanth, po)	0.502±0.020	9.242±0.120	146.0±0.760	57.62±0.278	178.05±3.208
CCl_4 (0.1ml/kg/day. ip)	2.440±0.109	5.931±0.052	2245.6±7.759	1394.4±1.59	403.61±1.496
CCl_4 +Silymarin (0.1ml/kg/day. ip + 100mg/kg/day.po)	0.507±0.001	8.890±0.0002	162.02±0.26	70.5±0.205	194.78±0.18
CCl_4+ methanolic stem bark extract. 0.1ml/kg/day. ip + 100mg/kg/day.po)	0.531±0.0001	8.127±0.0012	170.55±0.30	74.6±0.101	200.4±0.35
CCl_4 + methanolic stem bark extract (0.1ml/kg/day. ip + 200mg/kg/day.po)	**0.511±0.0211**	**8.975±0.0037**	**158.12±0.21**	**64.2±0.001**	**182.2±0.01**

[Values are expressed as mean ±SE. from 6 animals in each group]
AST=aspertate transaminase, ALT=alanine transaminase, ALP=alkaline phosphatase, N=six animals in each group.

TABLE 3: Molecular interactions of active constituents of *M. malabarica* with 1VKX receptor

Docked Molecule	No. of Hydrogen Bonds	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Hydrogen Bond Length	Lowest Binding Free energy (kcal/mol)	Reference RMSD
Biochanin A	03	1VKX:A:ARG169:HH:11	BIOCHANIN A: UNK0:O	2.132	-6.27	68.86
		BIOCHANIN A:: UNK0:H	1VKX:A:LEU136:O	2.138		
		BIOCHANIN A:: UNK0:O	1VKX:A:PHE16:O	1.941		
Prunetin	02	PRUNETIN:: UNK0:H	1VKX:A:VAL103:O	1.857	-6.03	69.61
		1VKX:A:ARG169:HH:11	PRUNETIN:: UNK0:O	2.104		
Malbaricone A	02	1VKX:A:ARG228:HH:12	MALABARICONE A:UNK0:O	1.944	-4.56	60.33
		1VKX:A:LYS507:HZ3	MALABARICONE A:UNK0:O	1.866		
Malbaricone B	04	MALABARICONE	1VKX:A:VAL226:O	1.853	-4.21	57.03
		B:UNK0:H 1VKX:A:LYS200:HZ3	MALABARICONE B:UNK0:O	1.785		
		1VKX:A:GLN229:HN	MALABARICONE B:UNK0:O	2.025		
		1VKX:A:GLN541:HE21	MALABARICONE B:UNK0:O	2.041		
Malbaricone C	04	MALBARICONES C::UNK0:H	1VKX:A:VAL226:O	1.889	-4.69	59.65
		MALBARICONES C::UNK0:H	1VKX:A:VAL226:O	2.111		
		1VKX:A:LYS203:HZ2	MALBARICONES C::UNK0:O	1.996		
		1VKX:A:GLN229:HN	MALBARICONES C::UNK0:O	2.193		

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Malbaricone D	03	MALBARICONES D::UNK0:H	1VKX:A:LYS476:O	2.064	**-6.49**	66.51
		1VKX:A:LYS507:HZ3	MALBARICONES D::UNK0:O	1.851		
		1VKX:A:ARG540:HE	MALBARICONES D::UNK0:O	1.98		
Silymarin	03	Silymarin::UNK0:H	1VKX:A:GLN229:OE1	2.167	**-5.59**	54.59
		Silymarin::UNK0:H	1VKX:A:ARG228:O	1.892		
		1VKX:A:ASN482:HN	Silymarin::UNK0:O	1.927		

ARG = Arginine, UNK = Unknown, LEU = Leucine, PHE = Phenyl alanine, VAL = Valine, LYS = Lysine, GLN = Glysine

Table 4: Molecular interactions of active constituents of *M. malabarica* with 1ILG receptor

Docked Molecule	No. of Hydrogen Bonds	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Hydrogen Bond Length in A^0	Lowest Binding Free energy (kcal/mol)	Reference RMS
Biochanin A	01	1ILG:A:GLN124:HE22	BIOCHANIN A:: UNK0:O	1.999	**-7.87**	65.99
Prunetin	01	1ILG:A:CYS123:HG	PRUNETIN:: UNK0:O	1.73	-6.89	72.92
Malbaricone A	01	MALABARICONE A:UNK0:H	1ILG:A:GLN124:OE1	2.187	-6.05	71.36
Malbaricone B	0				-4.83	69.45
Malbaricone C	01	MALBARICONES C::UNK0:H	1ILG:A:ASN243:ODI	2.057	-2.47	69.81
Malbaricone D	02	MALBARICONES D::UNK0:H	1ILG: A: MET82: O	2.064	-6.76	78.31
		1ILG:A:SER86:OG 1ILG:A:ARG249:HH:11	MALBARICONE D: UNK0: O	2.12		
Silymarin	00	--	--		0.9	75.05

GLN = Glysine, CYS = Cystine, ASN = Aspargine, MET = Methionine, SER = Serine, ARG = Arginine

Present study reveals that, hepatocellular necrosis caused by CCl_4 administration (Group II) leads to very high level of serum markers such as ALT (1394.4±1.59 IU/L), ALP (403.61±1.496 IU/L), AST (2245.6±7.759 IU/L) and Bilirubin (2.440±0.109 mg/dl), among these, alanine transaminase is a better index of liver injury as its activity represents 90% of total enzyme present in the body [28]. In the present investigation, the data in the table 2 indicates that, the animal groups treated with methanolic stem bark extract of *M. malabarica* at the dose of 200 mg/kg p.o. recorded significant reduction in the level ALT (64.2±0.001 IU/L), AST (158.12±0.21 IU/L), ALP (182.2±0.01 IU/L) and bilirubin (0.511±0.0211 mg/dl) compared to CCl_4 treated group and sylimarin treated group. The decrease in the serum transaminase indicates the stabilization of plasmamembrane and protection of hepatocytes against CCl_4 toxicity [29]. CCl_4, the inactive metabolite, is transformed to a free radical through the microsomal cytochrome P-450- dependent enzyme, resulting in activation of CCl_4 toxicity. Important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration and it has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid peroxidation induced by CCl_4 [30].

Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thereby help in preventing the free radical induced hepatic diseases [31]. Recent findings indicate that the liver protection and proliferation of hepatocytes accelerate in the presence of antioxidants [32]. Hence it is likely that the mechanism of hepatoprotection of *M. malabarica* is due to its antioxidant effect.

The ability of *M. malabarica* stem bark extract in ameliorating CCl_4 induced hepatic damage may be attributed to the active constituents viz., biochanin A, prunetin, malabaricone C, malabaricone A, malabaricone B and malabaricone D. *Insilco*

analysis revealed that these active constituents with lower E_{Total}, bind to the receptors involved in hepatoprotection, thus regulating the hepatoprotective activity. Binding energy for all the ligands is comparable to the standard drug silymarin.

As intended, the present study provides scientific evidence to the ethno medicinal use of this plant species used by the tribal group of Western Ghats in treating hepatitis. Based on the results of *Insilco* studies, it can be concluded that the lead molecules malbaricone D and Biochanin A with lowest binding energy could become strong candidates in designing the drug for hepatitis. Further studies in its mode of action, synergism, and drug likeness will be beneficial in drug designing for Hepatoprotection.

ACKNOWLEDGEMENTS

The first author is thankful to The Children's Education Society, Bangalore. Chairman Sri. Narasa Raju, Executive Director Sri. Ramesh Raju, The Principal, Prof. Nagaraj R, Prof. Kusum Paul, The Oxford College of Engineering, Bangalore, for their kind support and encouragement.

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