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Study of Hepatoprotective Activity from *Acanthus Ilicifolius* Leaves-The Combined Use of *in vitro*, *in vivo* and *In Silico* Analysis

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Abstract
Acanthus ilicifolius is a mangrove that is acting both ecological and medicinal importance, widely used in traditional systems of medicine, including Ayurveda and traditional Chinese medicine. *In vitro* studies revealed that the methanolic extract of *A. ilicifolius* leaves at a concentration of 500 µg/mL exhibited the free radical scavenging effect. In MTT assay the cell survival percentage was decreased along with increasing concentrations of the sample, IC_{50} values 63.403 µg/mL. *In vivo* HepG2 induced liver injury in mice exhibited a significant change in the levels of AST, ALT and ALP in the treated group and HCC alone significantly increased. The extract-treated group exhibited a reduction in serum creatinine levels that indicates no significant changes in renal function. Histopathology shows a decrease in the lobular inflammation and moderately maintains the liver architecture in control and migration of leukocytes into the inflamed area is significantly suppressed by the extract. The study included *in silico* analysis, in total 5 potential Hepatocellular Carcinoma (HCC) targets were predicted from 5 bioactive phytochemicals reported in the plant leaves. SEA and STITCH algorithms were used for target prediction and mined by CTD and PHARMGKB. In molecular docking, cyano colchicines showed the highest interaction with all the 5 targets, in par with standard drug sorafenib tosylate and doxorubicin. Collectively, the phytochemical present in *A. ilicifolius* leaves could be considered as a potential natural hepatoprotective agent.

Keywords
Acanthus ilicifolius; Hepatocellular carcinoma; Cyano colchicines; CDC25B; Traditional medicine.

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1. INTRODUCTION:
Hepatocellular carcinoma (HCC) is the fifth most common cancer in worldwide with a poor prognosis [1]. The common risk factors are alcoholism, hepatitis B and C infections, aflatoxin and cirrhosis of the liver. About three-quarters of the cases of this cancer are reported in East and Southeast Asia, including China, Korea, India and Japan [2].

A. ilicifolius, popularly known as 'Harkach Kanta' distributed widely throughout the mangroves of

India [3]. *A. ilicifolius* had been used as traditional Indian and Chinese medicine [4]. In general, *A.ilicifolius* had reported to have significant analgesic activity, antiviral, antibacterial and antifungal properties and used as a bush medicine by mangrove dwellers [5]. *A. ilicifolius* showed encouraging results in preventing liver cancer cells from progressing [6-8]. Computational target fishing is one of the major concerns in the drug design to identify the possible natural bioactive compounds in plants [9]. A computational approach of molecular docking was binding with protein active sites, were served as a major key methodology in the molecular drug design. It includes structural optimization and identifying biological activity through scoring functions by protein-ligand docking [10].

In our preliminary study [11]. *A. ilicifolius* methanolic leaf extract was exhibit 7 bioactive phytochemicals by GC-MS analysis. The *A. ilicifolius* leaf extract shows a maximum zone of inhibition against *Staphylococcus aureus* at 100 mg/mL concentration and the phytochemical 26.27-Di (nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy, which prevents colonization in *S. aureus* by blocking the Clumping factor B (ClfB) and Protein Translocase subunit (SecA) [12] and also reduce the liver stress, could have inhibited the activity of C-Jun N-terminal kinase 1 (JNK 1) thereby preventing the proliferation of hepatocytes [13]. The combined studies on *A. ilicifolius* leaves chemical constituents and anticancer activity has still not been systematically evaluated. The present study aimed to investigate the hepatoprotective function in *A. ilicifolius* leaves extracts through *in vitro*, *in vivo* and *in silico* studies.

2. MATERIALS AND METHODS:

2.1. Collection and preparation of the plant extracts

The fresh leaves of *A. ilicifolius* were collected in a mangrove forest of Parangipettai, Chidambaram, Tamil Nadu, India. The plant material was identified by the Botanical Survey of India, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India (BSI/SC/5/23/09-10/Tech.306). The fresh leaves of *A. ilicifolius* were washed and shade dried at room temperature (28 ± 2°C). The powdered sample was used for Soxhlet extraction with methanol solvent [14].

2.2. *In vitro* studies

2.2.1. DPPH scavenging assay

The plant extracts were prepared in different concentrations (100-500 µg/mL) to determine its ability to scavenging 2-2-diphenyl-2-picrylhydroxyl radicals (DPPH). DPPH solution (1mm of DPPH radical solution in 95% ethanol) of 800 µl was mixed with 200 µl of the sample. The mixture was vortexed well and incubated for 30 min at room temperature in dark. The absorbance of each sample was measured at 517 nm [15].

2.2.2. Total antioxidant capacity

Extract was prepared in different concentration with 1 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated in a thermal block at 95°C for 90 min and the absorbance of the aqueous solution of each tube was measured at 695 nm [16].

2.2.3. Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mm) was prepared with a standard phosphate buffer (pH=7.4). Different concentration of extracts in dilute water was added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min. The percentage inhibition of different concentrations of the extracts was determined and compared using standard ascorbic acid [17].

2.2.4. Cytotoxic activity

Cell growth inhibition studies were performed using the MTT assay. Cell viability was measured with the conventional MTT reduction assay. Briefly, HepG2 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h. The sample was prepared in various concentrations (0.1 µg – 100 µg/mL) of the test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10 µl, 5 mg/mL) at 37°C for 4 h with Dimethyl sulfoxide (DMSO) at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments [18].

2.3. *In vivo* studies

2.3.1. Experimental animals

BALB/c mice 4-6 weeks were used (23-30g weight), were purchased from Venkateshwara Breeding Institute, Bangalore, India. The animals were housed in ventilated plastic cages at 37±1°C, 40±10% humidity and 12 h light-dark cycles during the experimental period. All animal experimental protocols were reviewed and approved by the Ethics Committee of Karunya Institute of Technology & Sciences for the use of Laboratory Animals.

2.3.2. Experimental design

The animals were divided into four groups each group contains six animals (n=6), normal control, cancer control, HepG2 + standard drug and HepG2 + plant extract. Animals were induced with HepG2 cells received *A. ilicifolius* extract for a consecutive period of 15 days. HepG2 cells + standard drug group received doxorubicin (2.5 mg/kg of an animal) dissolved in phosphate buffer saline and administered orally for a consecutive period of 15 days.

2.3.3. Experimental induction of Hepatocellular carcinoma (HCC)

HepG2 cells were purchased in the National center for cell science (NCCS), Pune, India. The cells were counted under a hemocytometer and 4×10^6, suspended in sterile PBS and injected subcutaneously into the right flank of each mouse. After 1 week of cell line induction, the animals were used for the experimental study.

2.3.4. Administration of A. ilicifolius extract and standard drug

The plant extract was orally administered at a dosage of 500 mg/kg body weight of an animal by mixing it with PBS. The standard drug doxorubicin was used with freshly prepared solutions were obtained by dissolving doxorubicin in phosphate buffer saline at a dose of the 2.5 mg/kg body weight of an animal and are administered intraperitoneally [7].

Finally, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by cardiac puncture and serum was separated by centrifugation at 900 g for 10 min at 4^0C. The serum was collected and used for biochemical studies. The histopathology of the liver and kidney tissue was studied.

2.3.5. Biochemical parameters

2.3.5.1. Complete blood count

Beckman Coulter counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, color and density. The MAXM is a quantitative, automated, differential cell counter for *in vitro* diagnostic use. The MAXM measures these parameters in whole blood. The methods used to derive CBC parameters of counting and sizing, in combination with an automatic diluting and mixing device for sample processing and a single beam photometer for hemoglobinometry [19].

2.3.5.2. Study of hepatic profile

Enzymatic activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated spectrophotometrically using commercial diagnostic kits (Sigma-Aldrich, India).

2.3.5.3. Study of renal profile

Urea and creatinine levels in blood help to assess the function of kidneys. Jaffe's method is used for the quantitative determination of creatinine in serum (Sigma-Aldrich, India) [20].

2.3.5.4. Histopathological analysis

Internal organs including kidney and liver tissues were suspended in 10% formal saline for fixation preparatory to histological processing. The fixed liver and kidney tissues were sectioned embedded in paraffin and sections stained with Haematoxylin and Eosin (H&E). Light microscopic examination of multiple tissue sections from each organ in all groups was performed in all groups and the images representative of the histological profile were examined. Changes in the experimental histopathologic parameters for liver and kidney tissues were graded as follows: showing no changes, mild, moderate and severe changes, respectively, while the grading was also determined by percentage [21].

2.3.6. Statistical analysis

All the experimental data were expressed as mean ± SD. The significant difference from all experiment groups was assessed by one-way analysis of variance (ANOVA) using SPSS (IBM Corporation, Chicago, IL, USA). Values of $p < 0.05$ were considered as statistically significant.

2.4. *In silico* studies

2.4.1. Collection of ligands

Five major *A. ilicifolius* phytochemical compounds 26.27-Di(nor)-cholest-5,7,23-trien-22-ol,3-methoxy methoxy;9H –purin-6-amine, N, 9-bis (trimethylsilyl)-8-((trimethylsilyl) oxy); Cyanocolchicines; 3Beta-methoxy-5-cholesten-19-oic acid and Glycine, N-((3a, 5a, 12a)-oxo-3, 12-bis ((trimethylsilyl) oxy) cholan-24-yl), methyl ester was adopted as ligand [11]. Standard drug sorafenib tosylate and doxorubicin were used for the comparison.

2.4.2. Computational target fishing and data mining

The phytochemical compounds were used to predict the targets by similarity ensemble approach (SEA, http://sea.bkslab.org) [22] and STITCH (http://stitch.embl.de/) [23]. It detects the best mapping poses of query molecules the database annotated with target bank, binding DB, potential drug target databases and drug bank. Further, comparative toxicogenomics database (CTD, http://ctdbase.org/) and pharmacogenomics knowledge (PHARMGKB, http://www.pharmgkb.org) [24] were applied for target mining process.

2.4.3. Molecular docking

Ligand-protein docking was carried out to identify the interaction between the compounds and HCC targets using Auto Dock Vina and iGEMDOCK v2.1. The ligand structure data were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and the target proteins were retrieved from the protein data bank (PDB, https://www.rcsb.org/) [25].

3. RESULTS:

3.1. *In vitro* assays

3.1.1. DPPH scavenging assay

The antiradical activity of the plant extract was measured by scavenge DPPH free radicals and compared with the standard vitamin C. This activity

was increased in 500 µg/mL concentration of the sample (Fig. 1).

3.1.2. Total antioxidant capacity

The total antioxidant capacity was increased at 500 µg/mL in higher concentration and it compared with standard drug vitamin C (Fig. 2).

3.1.3. Hydrogen peroxide scavenging assay

The activity of the sample was dose-dependent, inhibition with hydrogen peroxide shows 80% scavenging activity at the concentration of 500 µg/mL, compared to the standard (Fig. 3).

3.1.4. Cytotoxic activity

The samples were prepared in various concentrations (0.001-100 µg/mL), the activity was significantly decreased in the cell viability of HepG2 cells in a dose-dependent manner (Fig. 4). The HepG2 cells exposed to extract at 100 µg/mL and higher concentrations were found to be a cytotoxic effect and the IC_{50} values were calculated to be 63.403 µg/mL. The standard drug doxorubicin was used for positive control.

3.2. In vivo experimental validation

3.2.1. Hepatic enzyme profile

The effect of *A. ilicifolius* leaf extract on the hepatic enzymes AST, ALT and ALP level was increased in the HepG2 group, compared to normal confirming the hepatotoxicity while in the sample groups AST, ALT and ALP activities were significantly reduced ($p < 0.01$, Fig. 5).

3.2.2. Renal Profile

The urea and creatinine levels were comparably reduced in the treatment group (Fig. 6). The extract treated animals showed a significant value of urea and creatinine ($p < 0.01$).

3.2.3. Complete blood count

Hematological parameters like RBC, WBC, Platelet count, packed cell volume (PCV), Mean corpuscular volume and Haemoglobin shows significant variation when compared with HepG2 control (Table 1).

3.2.4. Histopathological analysis

3.2.4.1. Liver

The normal microscopic architecture of the liver is composed of hexagonal lobules and maintained globular architecture. The portal tracts show unremarkable and individual hepatocytes expressed cytoplasmic vacuolation (Fig. 7A). Animals induced by HepG2 cells (HCC control), liver parenchyma shows lobular inflammation with foci, reactive atypia and sinusoids are mildly dilated and hepatocytes had binucleation (Fig. 7B). Plant extract-treated group shows, hepatocytes in binucleation and the portal tracts exhibit moderate periportal inflammation and bile duct hyperplasia, the liver parenchyma had globular inflammation (Fig.7C). Doxorubicin treatment group (2.5 mg/kg) exhibited the portal tracts in dense periportal inflammation, hepatocytes are normal and the central vein had congestion (Fig. 7D).

3.2.4.2. Kidney

In control, groups show normal architecture (Fig. 8A). HCC control group shows the mild tubular injury, glomeruli in mild mesangeal hypercellularity and congestion in the blood vessels (Fig. 8B). A plant extract-treated group shows mild injury in tubules, glomeruli and blood vessels had congestion (Fig. 8C). Doxorubicin (2.5 mg/kg) - treated group exhibited mild congestion in glomeruli and interstitium in inflammatory infiltrates with extravasated RBCs and Blood vessels had congestion (Fig. 8D).

3.3. *In silico* screening

3.3.1. Drug-Target prediction

The 5 pharmacophore models of *A. ilicifolius* were used to predict the potential targets using SEA and STITCH. SEA measures a similar reference target; it identifies the set of all the experimentally active compounds for the protein annotated in ChEMBL. For each reference, target SEA measures the similarity between the query compound and each reference compound and sums these numbers in Tanimoto Coefficients between ECFP4 fingerprints. To quantify the significance of this sum, SEA compares it to a background model fit with simulated targets made with compounds sampled at random from ChEMBL. Based on the disease specificity, 5 targets were mined by CTD and PHARMGKB (Table 2).

3.3.2. Molecular Docking

Five bioactive phytochemicals and 5 targets, which are responsible for HCC were taken for screening by iGemDock and AutoDock Vina. Cyano colchicines shows highest interaction among all other phytochemicals present in *A. ilicifolius* leaves extract with 3-oxo-5-alpha-steroid 4-dehydrogenase 1 (-142.84); M-phase inducer phosphatase 2 (-140.16); Steroid 17-alpha-hydroxylase/17, 20-lyase (-133.94); Sodium/bile acid cotransporter (-102.78) and Niemann-Pick C1 protein (-127.71), the results were compared with standard drug (Fig.9 and Supplementary Table 1).

Table 1 Complete Blood count

Contents	Normal	HCC control	HCC+Standard drug	HCC+Plant Extract
Total Hb (g/dl)	13.0	11.0	15.8	12.3
PCV (%)	39	33.7	50.6	37.5
WBC(×10^3/µL)	16.5	18.5	9.2	13.2
Lymphocytes (%)	90	75	93	88
Monocytes (%)	04	3	2	4
Eosinophils (%)	4	4	4	2
RBC (×10^6 /µL)	6.61	4.45	8.41	5.56
MCV (Fl)	59	60.2	60.1	71.5
MCH (Pg)	19.9	19.2	18.7	23.7
MCHC(g/dl)	33.8	27.7	31.2	32.8
Platelet count (×10^3/µL)	746	785	645	595
MPV (fl)	7.0	7.9	7.3	8.5

Table 2 Putative targets information of *A. ilicifolius*

ID	PDB ID	Gene name	Target
T01	4A2N	SRD5A1	3-oxo-5-alpha-steroid 4-dehydrogenase 1
T02	1CWR	CDC25B	M-phase inducer phosphatase 2
T03	2C17	CYP17A1	Steroid 17-alpha-hydroxylase/17,20 lyase
T04	3V5U	SLC10A1	Sodium/bile acid cotransporter
T05	3GKH	NPC1	Niemann-Pick C1 protein

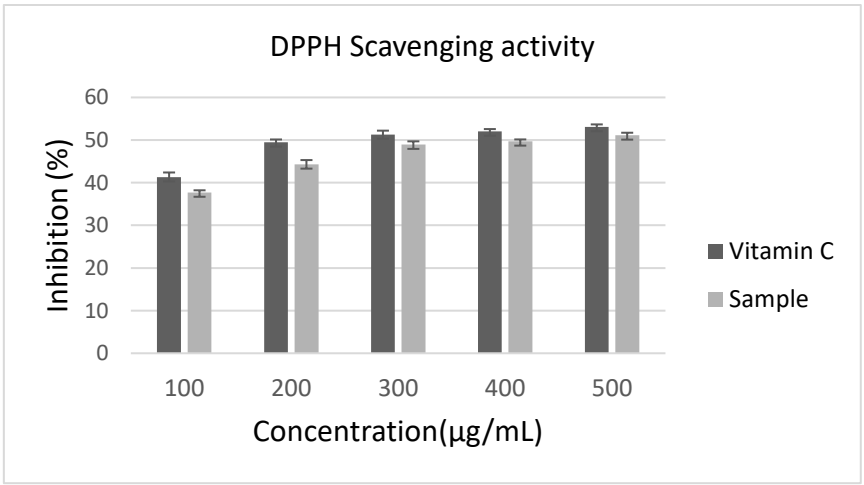

Fig.1. DPPH scavenging activities of *A. ilicifolius*

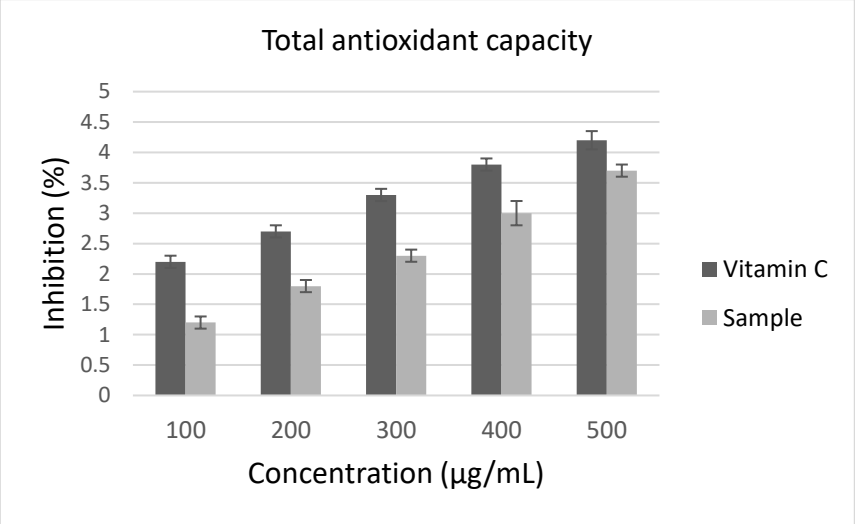

Fig.2. Total antioxidant capacity of *A. ilicifolius*

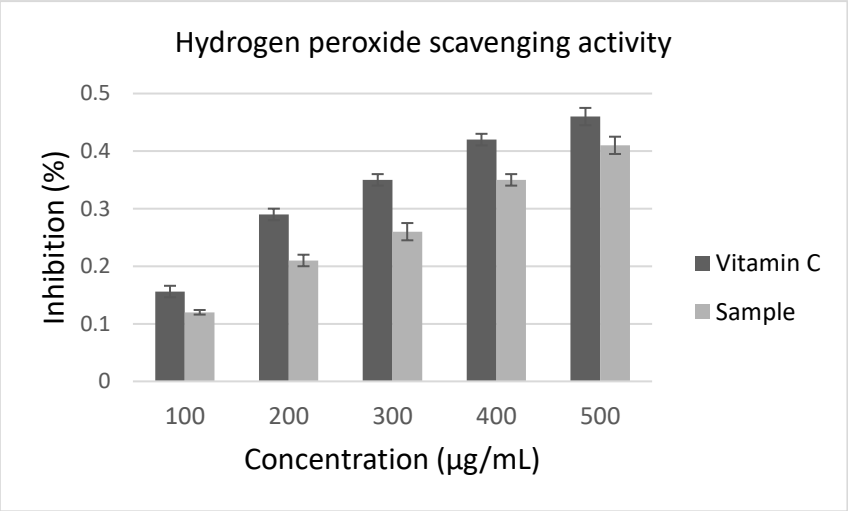

Fig.3. Hydrogen peroxide scavenging activities of *A. ilicifolius*

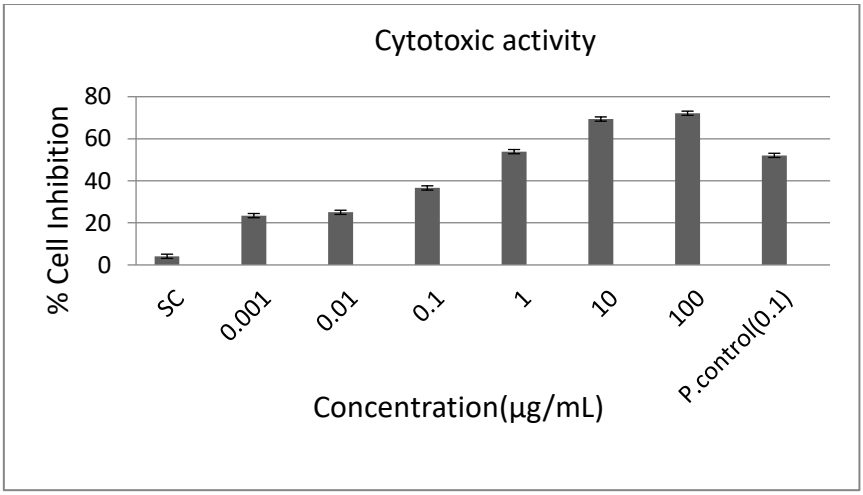

Fig.4. Cytotoxic activity of the sample in HepG2 cell
Notes: SC – Methanol; P. control - Doxorubicin

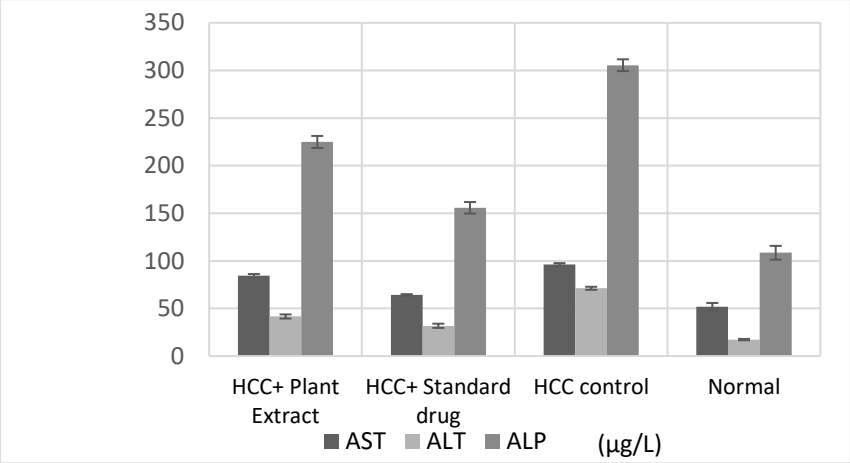

Fig.5. Effect of *A. ilicifolius* extract on Hepatic profile

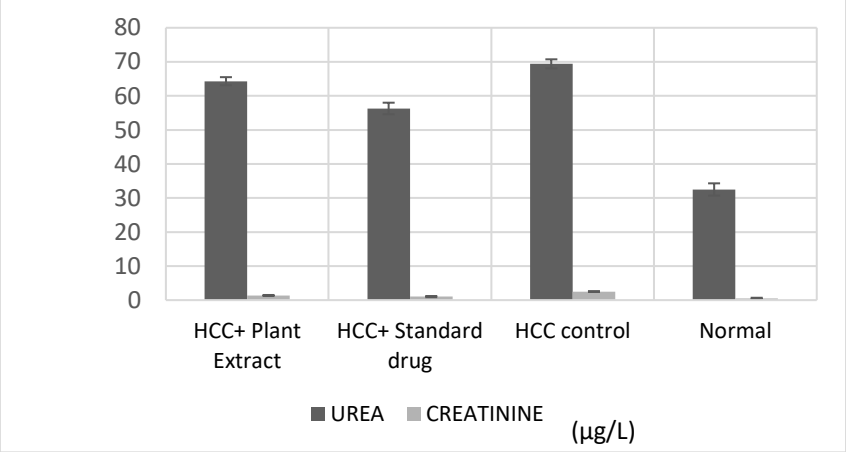

Fig.6. Effect of *A. ilicifolius* extract on renal profile

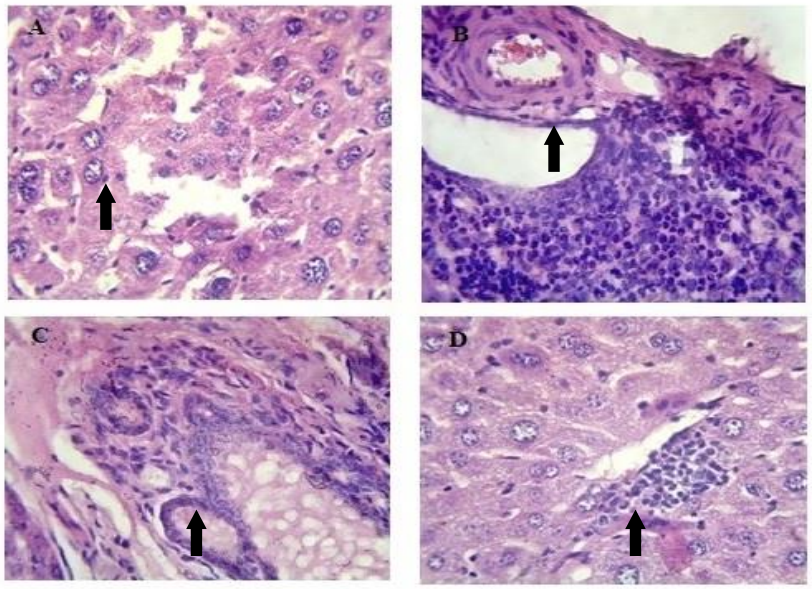

Fig.7. Histopathological liver (A) Normal liver maintained lobular architecture; (B) HCC control with lobular inflammation; (C) Induced HepG2 and plant extract shows bile duct hyperplasia; (D) Induced HepG2 and standard drug shows mild inflammation.

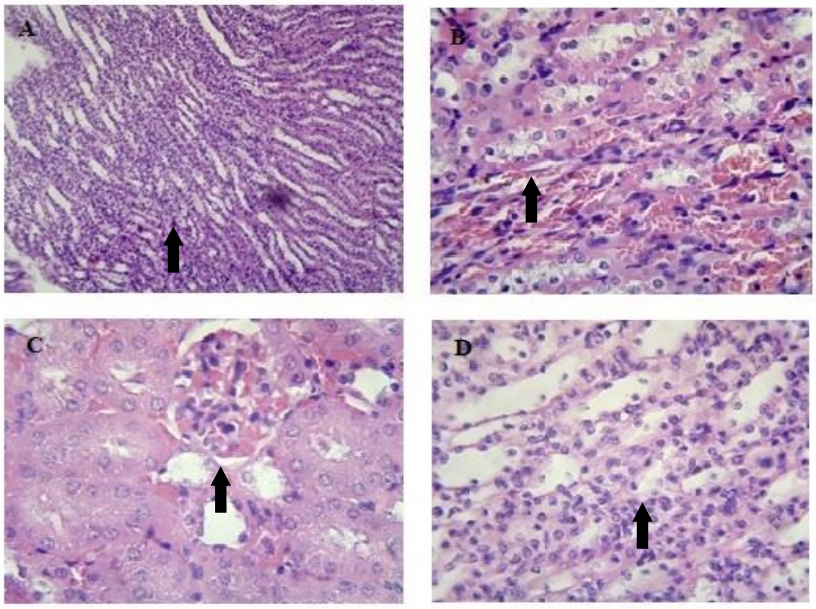

Fig.8. Histopathological kidney (A) Normal kidney interstitium; (B) HCC control group shows tubular injury with extravasated RBC's; (C) Induced HepG2 and plant extract shows mild glomerular congestion; (D) Induced HepG2 and standard drug shows interstitium with inflammation.

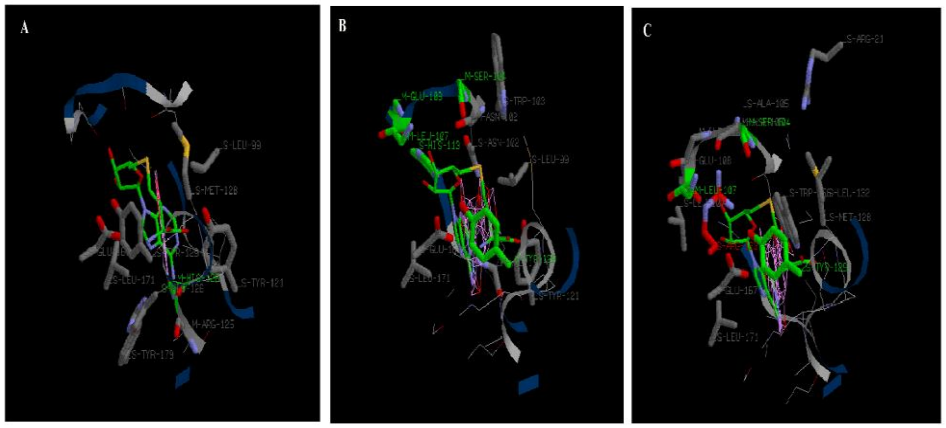

Fig.9. Hydrogen bond interaction with ligand and protein (A) Cyano colchicines with 3-oxo-5-alpha-steroid 4-dehydrogenase 1; (B) Doxorubicin with 3-oxo-5-alpha-steroid 4-dehydrogenase 1; (C) Sorafenib tosylate with 3-oxo-5-alpha-steroid 4-dehydrogenase 1.

Supplementary Table 1. Interaction of A. ilicifolius bioactive compounds with HCC targets

		iGemDock				AutoDock Vina	
Compounds	**PDB ID**	**Energy**	**VDW**	**H-bond**	**Elec**	**Binding affinity**	**RMSD value**
26.27-Di (nor)-cholest-5, 7, 23-trien-22-ol, 3-methoxymethoxy	4A2N	-82.44	-76.4	-6.04	0	-5.6	1.836
	1CWR	-83.25	-80.75	-2.5	0	-6.5	1.388
	2C17	-99.47	-94.47	-5	0	-8.5	0.99
	3V5U	-69.36	-69.36	0	0	-1.4	1.538
	3GKH	-93.69	-78.24	-15.44	0	-5.6	1.464
	4A2N	-76.44	-64.13	-12.31	0	0	0
	1CWR	-69.58	-69.58	0	0	0	0

9H –purin-6-amine, N, 9-bis (trimethylsilyl)-8-((trimethylsilyl) oxy)	2C17	-74.79	-67.79	-7	0	0	0
	3V5U	-61.64	-49.97	-11.66	0	0	0
	3GKH	-72.57	-64.06	-8.51	0	0	0
	4A2N	-142.84	-135.25	-7.59	0	-7.6	1.299
	1CWR	-140.16	-128.68	-11.48	0	-7.1	1.486
Cyano colchicines	2C17	-133.94	-126	-7.94	0	-11	1.186
	3V5U	-102.78	-88.31	-14.48	0	-5.3	1.63
	3GKH	-127.71	-106.36	-21.35	0	-7.9	1.801
	4A2N	-68.82	-62.76	-3.5	-2.57	-5.6	1.429
	1CWR	-83.36	-83.36	0	0	-6.2	1.269
3Beta-methoxy-5-cholesten-19-oic acid	2C17	100.57	-86.44	-14.13	0	-8.2	2.978
	3V5U	-67.6	-62.45	-5.15	0	-4.9	1.665
	3GKH	-72.42	-72.42	0	0	-4.4	1.488
	4A2N	-81.15	-72.1	-9.06	0	0	0
	1CWR	-94.65	-91.63	-3.02	0	0	0
Glycine, N-((3a, 5a, 12a)-oxo-3, 12-bis ((trimethylsilyl) oxy) cholan-24-yl), methyl ester	2C17	-149.39	-129.05	-17.91	-2.44	0	0
	3V5U	-67.6	-55.85	-12.22	0.47	0	0
	3GKH	-97.73	-82.27	-15.46	0	0	0
	4A2N	-155.89	-135	-20.89	0	-8.7	1.614
	1CWR	-154.72	5	0	0	-7.8	1.328
Doxorubicin	2C17	-181.66	-158.78	-22.88	0	-11.5	2.519
	3V5U	-123.85	-85.81	-38.04	0	-4.3	1.712
	3GKH	-141.96	-116.64	-25.32	0	-7.4	1.87
	4A2N	-155.9	-130.43	-22.94	-2.52	-5.1	2.285
	1CWR	-136.91	-127.9	-7	-2.01	-5.2	19.322
Sorafenib tosylate	2C17	-185.35	-164.4	-17.02	-3.93	-6.7	0.957
	3V5U	-115.45	-110.87	-4.58	0	-5.7	1.564
	3GKH	-135.96	-117.59	-18.37	0	-6.1	2.238

4. DISCUSSION:

Phytochemicals are capable of providing longer remissions and perhaps a complete cure for many diseases. Most of the biochemical molecular mediators involved in hepatocellular carcinogenesis can be targeted by natural products. The plant extract in the antioxidant assay and cytotoxic assay shows potent activity in par with the standard drug, it assumes that the presence of various biochemical compounds of *A. ilicifolius* leaves might protect the release of toxic substances. HCC is reported to be associated with the decreased Hb content because of reduced erythrocyte deformability. The reduced deformability leads to a shortening of the erythrocyte lifespan [26]. The decreased Hb level is frequently seen in HCC and other liver damage. The serum showed an increase in the platelet count. The WBC count also increased in the serum to destroy the invading pathogens. HCC generally results in the accumulation of leukocytes; results show the migration of leukocytes into the inflamed area is significantly suppressed by the plant phytochemicals [27]. Histopathology in mice, an increase in binucleated cells may be due to lipolytic characteristics of parathinon that alter the permeability of cell membranes of the hepatocytes [28]. Sample decreases the lobular inflammation and moderately maintains liver architecture.

In silico studies, the bioactive phytochemical was effectively bind with all the HCC targets. Especially,

cyano colchicines had maximum interaction with 3-oxo-5-alpha-steroid 4-dehydrogenase 1; M-phase inducer phosphatase 2; Steroid 17-alpha-hydroxylase/17, 20 lyase; Sodium/bile acid cotransporter and Niemann-Pick C1.

M-phase inducer phosphatase 2 (CDC25B) CDC25 phosphatases are the main regulator in the eukaryotic cell cycle and it activates the Cdk/cyclins, overexpression, and poor prognosis may cause the many diverse cancers. The CDC25 has three isoforms in human CDC25A, CDC25B, and CDC25C [29]. CDC25A and CDC25B, as an oncogene, are overexpressed in various cancer, including head and neck cancer, non-small cell lung cancer, gastric cancer, colon cancer, esophagus cancer, breast cancer and ovarian cancer [30]. The previous records [31] used 200 liver tissue samples for the gene expression profiles and identified CDC25B as one of the most significantly over-expressed genes in HCC compared to non-tumor liver. It could provide the CDC25 phosphatases are attractive targets for anticancer drug development.

Steroid 17-alpha-hydroxylase/17, 20-lyase (CYP17A1), also known as cytochrome P450c17 or CYP17, is the microsomal p450 enzyme that catalyzes both 17 a-hydroxylase and 17, 20-lyase activities which involved in the biosynthesis of steroid hormones [32]. CYP17A1 is a main preliminary target involving prostate cancer proliferation [33].

Sodium/bile acid cotransporter, Bile acids (BA) are synthesized via a neutral pathway and acidic pathway [34]. The majority (75%) of the pathway in hepatocytes were synthesized neutral way [35]. Bile acids initiate the activation of the oncogenic mTOR pathway in the liver and it associated with oncogenesis in human cervical and colon cancers [36].

Niemann-Pick C1 protein (NPC1L1) is expressed in the human liver and intestine, involving in intestinal cholesterol absorption in humans [37]. Adeno-associated virus 8 (AAV8) to deliver NPC2 into the liver and evaluated its inhibition effects in spontaneous HCC mouse model. The data demonstrated that AAV-NPC2 treatment not only reduced the tumor incidence but also decreased the inflammatory infiltration and early HCC marker expression in mice. Therefore, the study suggests NPC2 may play an important role in negatively regulating liver cancer development and that targeted expression [38].

5. CONCLUSION:

Safety is important, to concern developing the new drugs, animal testing, it has been the most precise and consistent method for drug discovery, still is time-consuming, expensive and also some side effects due to the animal's and humans' differences. In this case, drug trials possess the problematic conditions, for this reason, the combined methods of *in vitro*, *in vivo* and *in silico* help to enable the development of better and safer medicines. The *A. ilicifolius* leaves show the presence of alcohol and a free hydroxyl group shows the presence of phytosterols. Further, the study extended in *A. ilicifolius* leaves and identified three compounds. Namely, cholest-5-en-3-ol(3,Beta.)-, carbonochloridate; cholesterol and cholest-5-en-3-ol(3, Beta.)-, propionate. In the future, the study focused on selective compounds to identify the mechanism of liver damage.

6. REFERENCES:

[1] Raza A, Gaga, Sood, K., Hepatocellular carcinoma review: current review and evident based medicine. World Journal of Gastroenterology 2014; 20(15): 4115-4127.

[2] Asghar U, Meyer T. Are there opportunities for chemotherapy in the treatment of hepatocellular cancer? J Hepatol 2012; *56*: 686-695.

[3] Thirunavukarasu P, Ramanathan, Ramkumar L. Haemolytic and anti-microbial effect in the leaves of *Acanthus ilicifolius*. Journal of Pharmacology and Toxicology 2011; 6(2):196-200.

[4] Amritpal S, Sanjiv D, Ashish S. *Acanthus ilicifolius* linn-lesser known medicinal plants with significant pharmacological activities. International Journal of Phytomedicine 2009; 1: 1-3.

[5] Abeysinghe PD. Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian Journal of Pharmaceutical Sciences 2010; 72: 167-172.

[6] Singh D, Veri V. Phytochemical and pharmacological potential of *Acanthus ilicifolius.* Journal of Pharmacology and Bioallied sciences 2013; 5(1): 17-20.

[7] Babu BH, Shylesh BS, Padikkala J. Tumour reducing and anticarcinogenic activity of *Acanthus ilicifolius* in mice. Journal of Ethnopharmacology 2001; 79 (2002): 27-33.

[8] Zhao P, Li-Chen and Lin-Hu Li. SC-III3, a novel scopoletin derivative, induces cytotoxicity in hepatocellular cancer cells through oxidative DNA damage and ataxia telangiectasia-mutated nuclear protein kinase activation. BMC cancer 2014; 14: 987.

[9] Gambari R. Predictive analyses of biological effects of natural products: from plant extracts to biomolecular laboratory and computer modeling. Evidence-Based Complementary and Alternative Medicine. Article ID 383290, 4pages, https://doi.org/10.1093/ecam/nep096.eCAM, 2011.

[10] Sousa SF, Fernandes PA, Ramos MJ. Protein-ligand docking: current status and future challenges. Proteins 2006; 65: 15-26.

[11] Ganesh S, Vennila JJ. Phytochemical analysis of *Acanthus ilicifolius* and *Avicennina officinalis*.

Research Journal of Phytochemistry 2011; 5 (1): 60-65.

[12] Ganesh S, Jannet Vennila J, Annie Mercy A. Effects of Phytochemicals extracted from *Acanthus Ilicifolius* against *Staphylococcus aureus:* An *In-silico* approach. American Journal of Drug Discovery and Development 2013; 3 (4): 293-297.

[13] Mano Bala J, Jannet Vennila J, Ganesh S, Sachidan Singh. Analysis of active components in *Acanthus ilicifolius* leaves for their potentialchemoprotective activity against hepatocellular carcinoma: An *in-silico* approach. Journal of Pharmacy Research 2011; 4(6): 1809-1812.

[14] Cumpson P, Sano, Naoko. Stability of reference masses V: UV/ozone treatment of gold and platinum surfaces. Metrologia 2013; 50 (1): 27-36.

[15] Mukund S, Sivasubramanian V, Senthil K. *In vitro* antioxidant activity of methanolic extract of *Oscillatoria terebriformis* C. An Agardh ex Gomont. Journal of Algal Biomass 2013; 4(1): 17-25.

[16] Shirwaikar A, Prabhu KS, Punitha ISR. *In vitro* antioxidant studies of phaeranthus indicus (Linn). Indian J Exp Biol 2006; 44: 993-996.

[17] Jayaprakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models. J. Agric Food Chem 2001; 55:1018-1022.

[18] Evelyn ML, Pina, Fernando WC, Araújo, Ivone A, Souza, Isla VGA, Bastos, Teresinha G, Silva, Silene C, Nascimento, Gardenia C, Militão, Luiz AL, Soares, Haroudo S, Xavier, Sebastião J Melo. Pharmacological screening and acute toxicity of bark roots of Guettarda platypoda. Brazilian Journal of Pharmacognosy 2012; 1(3): 1-8.

[19] Corash L. Platelet Sizing: Techniques, Biological Significance, and Clinical Applications. Current Topics in Hematology. Alan R. Lise, Inc, New York, 1983.

[20] Amritpal Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandou RJ, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES. Large-scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science 1998; 280: 1077-1082.

[21] Seri S, Ercan F, Gedik N, Yüksel M, Alican I. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. Toxicology 2007; 230: 256–264.

[22] Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. Nat. Biotechnol 2007; 25: 197-206.

[23] Kuhn M, Szklarczyk D, Franceschini A, Von mering C, Jensen LJ, Bork P. STITCH 3: zooming in on protein-chemical interactions. Nucleic Acids Res 2012; 40: 876-880.

[24] Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF et al. Pharmacogenomics knowledge for personalized medicine. Clin. Pharmacol. Ther 2012; 92: 414-417.

[25] Shen J, Tan C, Zhang Y, Li X, Li W, Huang J, Shen X, Tang Y. Discovery of Potent Ligands for Estrogen Receptor β by Structure-Based Virtual Screening. J. Med. Chem 2010; 53: 5361-5365.

[26] Clive D, Tucker EW, Hajian G. Non-clinical Toxicology studies with Zidovudine-Gnetic Toxicity tests and Carcinogenecity Bioassays in Mice and Rats. Fundamental and applied Toxicology 1996; 32: 152-158.

[27] Pranca AVC, Elias J. Diagnosis, Ageing and treatment of Hepatocellular carcinoma. Brazilian Journal of Medical and Biological Research 2004; 37: 1689-1705.

[28] Schalgether M, Terracicano, L Sorrentino P. Histopathology of Hepatocellular Carcinoma. World Journal of Gastroenterology 2014; 20(43): 15955-15964.

[29] Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol 2006; 18:185-91.

[30] Kristjansdottir K, Rudolph J. Cdc25 phosphatases and cancer. Chem Biol 2004; 11:1043-1051.

[31] Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D, Brown PO. Gene expression patterns in human liver cancers. Mol Biol Cell 2002; 13:1929-1939.

[32] Auchus RJ, Lee TC, Miller WL. Cytochrome b5 augments the 17, 20-lyase activity of human P450c17 without direct electron transfer. J. Biol. Chem 1998; 273: 3158–3165.

[33] Petrunak EM, DeVore NM, Porubsky PR, Scott EE. Structures of human steroidogenic cytochrome P450 17A1 with substrates. J. Biol. Chem 2014; 289: 32952–32964.

[34] Zhu Y, Liu H, Zhang M, Guo GL. Fatty liver diseases, bile acids, and FXR. Acta Pharm Sin B 2016; 6(5): 409–412.

[35] Asgharpour A, Kumar D, Sanyal A. Bile acids: emerging role in management of liver diseases. Hepatol Int 2015; 9(4):527–533.

[36] Dufour M, Faes S, Dormond-Meuwly A, Demartines N, Dormond O. PGE2-induced colon cancer growth is mediated by mTORC1. Biochem Biophys Res Commun 2014; 451:587–591.

[37] Storch J, Xu Z. Niemann-Pick C2 (NPC2) and intracellular cholesterol trafficking. Biochim Biophys Acta 2009; 1791: 671–678.

[38] Yi-Jen Liao et al. Niemann-Pick type C2 protein regulates liver cancer progression via modulating ERK1/2 pathway: Clinicopathological correlations and therapeutical implications. Int. J. Cancer 2015; 137: 1341–1351.