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Research Article

Evaluation of Anti-Tumour Potential of *Acanthus ilicifolius* (Linn.) in HepG2 Cell Line Induced Hepatocellular Carcinoma in Mice

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ABSTRACT

Acanthus ilicifolius has long history of medicinal plant belong to the family Acanthaceae. In the present study, ethanolic extract of *A. ilicifolius* showed antitumour potential against HepG2 cell line induced hepatocarcinoma in mice. The phytochemical analysis were studied by using strandard methods. The heptocarcinoma mice were orally treated with extract of *A.ilicifolius* (500 mg/kg B.W). The result indicates the presence of phytoconstituents such as Protein (2.5%), Carbohydrates (0.16%) , alkaloids (0.42%), flavonoids (0.62%), saponins (4.5%), tannins (1.0%) and terpenoids (0.20%). In FTIR spectrum of test sample, the strong absorbance were showed at peak 678.94 cm^{-1}, 1514.12 cm^{-1},1741.72 cm^{-1}, 2304.94 cm^{-1}, 3597.24 cm^{-1}, 3728.40 cm^{-1} which are associated with presence of functional groups such as Mono substituted benzene, aromatic (c=c), aldehyde (c=o), disubstituted alkynes(c=c), alcohols with free hydroxyl group (O-H), alcohols, phenols (O-H). Treatment effectively suppressed HepG2 cells induced mice as revealed by decrease in the levels and extend of Alanine transaminase (84.3±1.86), Aspartate transaminase (41.6±2.15), Alkaline phosphatase (224.9±6.3), Urea (64.3±1.20) and creatinine (1.36±0.155). Histopathological analysis of liver and kidney was also evaluated. This study demonstrates that the *A. ilicofolius* have a potent anticancer Properties.

Keywords: *Acanthus ilicifolius,* HepG2 cells, Hepatocellular Carcinoma, Anticancer.

INTRODUCTION

Cancer is one of the most severe health problem in both developing and developed countries, worldwide. Among the most common (lung, stomach, colorectal, liver, breast) types of cancers, lung cancer has continued to be the most common cancer diagnosed in men and breast cancer is the most common cancer diagnosed in women. An estimated 14 million people were diagnosed with cancer across the world in 2015, and 7.6 million people died from the cancer during the same year. Lung cancer, breast cancer, colorectal cancer and stomach cancer accounted for two-fifths of the total cases of cancers diagnosed worldwide. More than 70% of all cancer deaths occurred in low and middle income countries. Deaths due to cancer are projected to continuously increase and it has been estimated that there will be 11.5 million deaths in the year 2030 and 27 million new cancer cases[1].

Hepatocellular carcinoma (HCC) is one of the malignancies with increasing incidence. Though there have been several curative methods for the disease, but the survival solely depends on the tumour location and the underlying liver disease, cirrhosis. There has been urgent need for the treatment of HCC to prevent its occurrence or its reoccurrence. Herbal compounds are known to play a major impact in all the stages of HCC. Therefore, there has been an increase in the research for the use of plant derived compounds as potential anticancer agents against HCC for a novel drug development[2].

Chemotherapy as well as conventional treatment for the cure of cancer causes the adverse and toxic side effects therefore fails to control the cancer disease. The alternate solution for the harmful effects of synthetic agents is the use of medicinal plants[3]. The medicinal plants contain chemical constituents of therapeutic value[4]. These chemical substances produce physiological action on the human body. It has been shown currently by clinical studies and phytochemical compared to synthetic chemicals[5]. Plants are the effective source of anticancer agent and over 60% anticancer agents are derived from natural resources[6]. Although number of anticancer agents are derived from medicinal plants but still there is a number of plants that exhibits anticancer potential but they have not yet been fully investigated such as mangroves plants.

Mangroves are biochemically unique, producing a wide array of novel natural products. Substances in mangroves have long been used in folk medicine to treat diseases. Although the chemical constituents of most mangrove plants still have not been studied extensively, for chemotherapeutic agents. *Acanthus illicifoilis* L. is a common mangrove plant and is used in several countries to treat different diseases. Previous studies Mastaller *et al*[7] have confirmed that some of the species produce compounds that can exert some pharmacological, antimicrobial and anti - inflammatory activities. The aim

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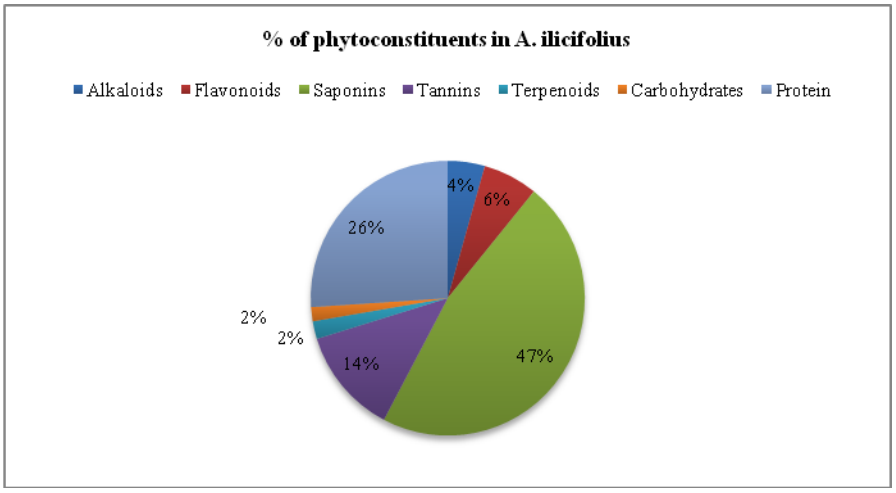

Figure 1: Phytochemical analysis *A. ilicifolius.*

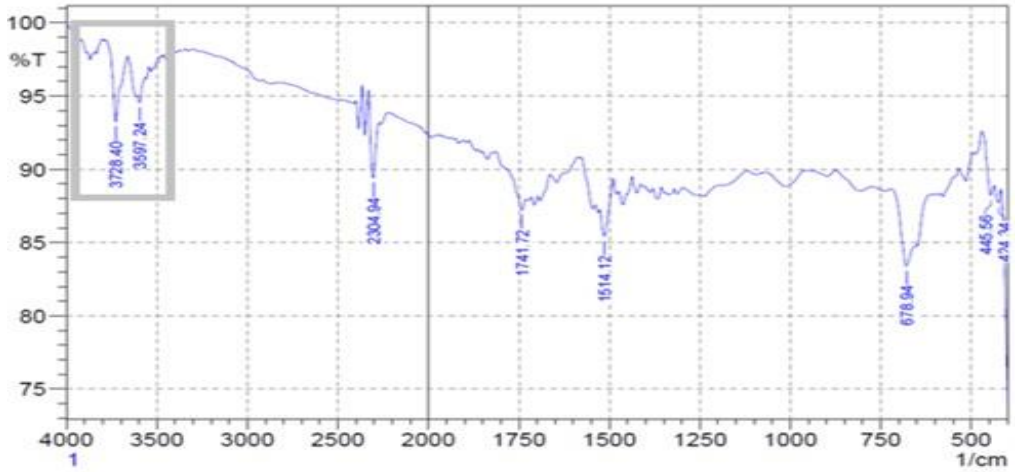

Figure 2: FTIR analysis of ethanolic extraction of *A. ilicifolius.*

of the present study is to evaluate the anti-tumor effect of whole leaf extract of *A. ilicifolius* in HepG2 cell line induced Hepatocellular carcinoma in mice.

METERIALS AND METHODS

Collection and Authentication of Experimental Plant

Fresh leaves of the selected plant *A. ilicifolius* were collected from Muthupet, India. The herbarium number of the plant is (BSI/SC/5/23/09-10/Te). The plant was identified by Botanical Survey of India.

Preparation of Extraction

The coarse powder of plant material was extracted with ethanol by using soxhlet apparatus. The excess ethanol was evaporated by using vacuum (IKA ® RV 10) rotory evaporator.

Phytochemical Screening

Ethanolic leaf extraction of *A. ilicifolius* were subjected to preliminary phytochemical screening was followed by standard methods[8-9].

FT-IR Analysis

The extract was replaced by distilled water each time. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried extract were analyzed by ALPHA FT-IR Spectrometer (from Bruker, Germany) for the detection of different functional groups by showing peaks from the region of 4000 $cm^{-1}$to 500 cm^{-1}.

Animals

They were kept in plastic animal cages at animal house maintained at standard temperature and humidity with 12 h light and dark cycle. The animals were fed with standard pellet diet and water. The animals are handled according to Good Laboratory Practice (GLP). After one week of acclimatization, the animals were used for further research experiments. Ethical clearance was obtained from institutional animal ethical committee (CPCSEA/265/2015) and conducted according to Indian national science academy guidelines for the use and care of experiments.

The animals were divided into four groups of six animals each

Group 1: Normal Control

Group 2: HepG2 cells at 4×10^6cells

Group 3: Treated with *A. ilicifolius* (500 mg/kg B.W)

Group 4: Treated with Doxorubicin (2.5 mg/kg B.W)

Acute Toxicity Study

Acute toxicity study of the extract of *A. ilicifolius* was performed using swiss albino mice according to OECD guidelines no. 425. The extract was administered orally at doses 150, 250, 350, 450 and 500 mg/kg of body weight.

Table 1: FTIR analysis of *A. ilicifolius.*

S.no	Peaks	Functional groups
1.	678.94	Mono substituted Benzene
2	1514.12	Aromatic (c=c)
3	1741.72	Aldehyde (c=o)
4	2304.94	Disubstituted alkynes(c=c)
5.	3597.24	Alcohols with Free hydroxyl group(O-H)
6.	3728.40	Alcohols, Phenols(O-H)

The control group received only the normal saline (10 mL/kg b.w).

Induction of Hepatocellular Carcinoma

HepG2 cells (NCCS, Pune) were counted under haemocytometer and 4×10^6 cells were suspended in sterile PBS and injected subcutaneously into the right flank of each mice. After 1 week of cell line induction, the animals were used for experimental study.

Sample Collection

After the experimental study the animals were sacrificed by cervical dislocation under mild chloroform as aesthesia. Blood was collected by cardiac puncture and serum was separated by centrifugation for 10 minutes at 3000 rpm. The serum was then collected and used for biochemical studies. The histopathology of the liver and kidney tissue was studied.

Biochemical studies

Study of hepatic profile

Alkaline phosphatase (ALP), Aspartate transferase (AST) and Alkaline aminotransferase (ALT) estimated by using standard method[10].

Study of Renal Profile

The study of renal profile performed by using standard methods, Urea[11] and Creatinine[12].

RESULTS

The preliminary phytochemical screening results of *A. ilicifolius* showed (fig-1) various bioactive secondary metabolites constituents such as Protein (2.5%), Carbohydrates (0.16%) , alkaloids (0.42%), flavonoids (0.62), saponins (4.5%), tannins (1.0%), terpenoids (0.20%).

The extraction of *A. ilicifolius* leaves was done. The leaves were also separately processed to extract the sterol which was characterized using FTIR (Fig-2). The FTIR spectrum of *A. Ilicifolius* extract was observed peaks at 678.94 cm^{-1}, 1514.12 cm^{-1},1741.72 cm^{-1}, 2304.94 cm^{-1}, 3597.24 cm^{-1}, 3728.40 cm^{-1} which are associated with presence of functional groups is mono substituted benzene, Aromatic (c=c), Aldehyde (c=o), Disubstituted alkynes(c=c), Alcohols with Free hydroxyl group(O-H), Alcohols, Phenols(O-H). At peaks 3597.24 cm^{-1} and 3728.40 cm^{-1} illustrates a free hydroxyl group which confirms the presence of sterols.

No significant changes were observed in the behavioural or autonomic responses in mice after treatment with different dosses of ethanolic leaf extract of *A. ilicifolius.*

The effects of *A. ilicifolius* leaf extract on AST, ALP and ALT in HepG2 cell line induced mice is shown in the table 2. There is an extreme significant AST level observed between the healthy control and experimental

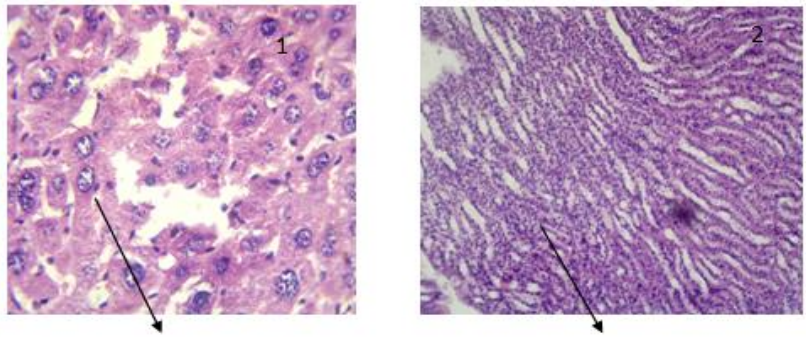

Figure 3: Group 1-Normal group.

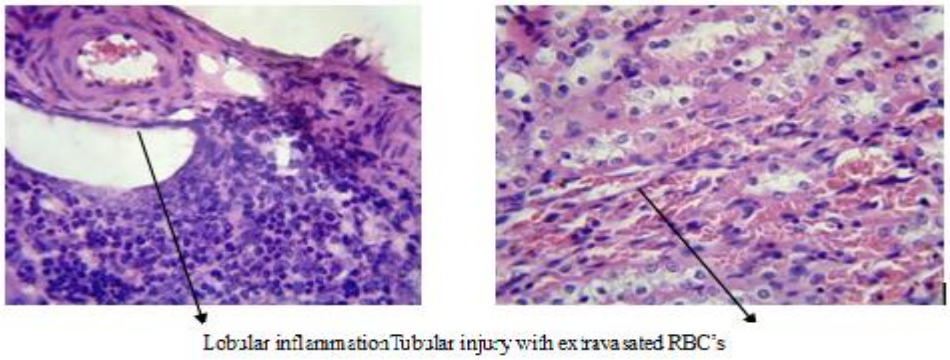

Figure 4: Group- 2 HepG2 control.

mice, it showed a significant value P<0.01 and the S.D value 84.3±1.86 respectvely. The ALT level were observed between the treated extract and HepG2 control mice with P<0.01 and S.D value 41.6±2.15. There is an extreme significant ALP level observed between the treated extract and HepG2 control mice with P<0.01 and S.D value of 224.9±6.3.

The effects of *A. ilicifolius* on urea and creatinine in HepG2 cell line induced mice was shown in the table 3. The extract treated animals showed a significant value (P<-0.01) and the S.D value 64.3±1.20 and creatinine is P<0.01 and S.D value 1.36±0.155. However, the result did not show significant value with the drug Doxorubicin.

In Histopathological sections of liver and kideny shows maintained globular architecture. Individual hepatocytes show cytoplasmic vacuolation. The kidney shows normal cortex and medulla. The glomeruli shows normal morphology.

In group 2 animals the liver parenchyma shows lobular inflammation with foci and reactive atypia. Sinusoids are mildly dilated. Hepatocytes show binucleation which is given in fig 4 and the tubules show mild tubular injury. The glomeruli shows mild mesangeal hypercellularity. Blood vessels show congestion.

In Group 3 animals liver portal tracts show dense periportal inflammation. Hepatocytes are remarkable. The central vein shows congestion. In fig.5, the glomeruli shows mild congestion. The interstitium shows inflammatory infiltrates with extravasated RBC's. Blood vessels show congestion.

In group 4 the hepatocytes show binucleation. The portal tracts show moderate periportal inflammation and bile duct hyperplasia. The liver parenchyma shows globular inflammation which is given in the fig 6. In kidney the tubules show mild injury. The glomeruli show mild congestion. Blood vessels show congestion.

DISCUSSION

Medicinal plants are the major source of therapeutic agents to cure human diseases. Recent researches in medicinal and aromatic plants made the health-care enhancement for the purpose of humankind. The vast floral resources of mangrove are best known for their medicinal properties and studies have been made on mangrove forest plants and their bioactive compounds during these days due to the medical importance. The mangrove plant extracts have been practiced as a common method for the treatment of health disorders for many centuries. The bioactive compounds of mangrove plants are unique in their actions. Since they possess competence in many bioactive principles against disease producing microbial organisms[13]. The secondary metabolites such as Protein, Carbohydrates, alkaloids, steroids, phenols, and terpenoids have been chemically characterized from mangroves which have toxicological, pharmacological, and ecological importance. This study reveals that ethanolic extract of *A. ilicifolius* exhibited the presence of alkaloids, terpenoids, flavonoids, phenol, tannins, phytoserol, carbohydrates and saponins. In phytochemical analysis the major compounds of alkaloids, phenols, tannins and etc., are rich in medicine and constitute most of the valuable drugs. They have physiological effect on animals[14].

Infrared (IR) spectroscopy is among the most powerful spectroscopic techniques for compound analysis since it covers the details on the functional group as well as chemical composition that are contained in the infrared spectrum of specific substances[15]. Infrared spectroscopy is a powerful method for studying molecular structure and intra molecular interaction in biological tissues and cells[16]. The FTIR analysis of plant leaf extract shows the presence of the functional groups of alcohol and a free hydroxyl group indicating the presence of sterol.

The Aspartate transaminase (AST) is the enzyme responsible for catalysing the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate in the liver, it is present in the liver parenchymal cells. Alkaline phosphatise is a hydrolase enzyme in the cells lining the biliary ducts of the liver, it performs dephosphorylation. Alanine transaminase (ALT) is an enzyme present in hepatocytes and it catalyses the transfer of an amino group from alanine to α-keto glutarate, the products of this reversible transamination reaction being pyruvate and glutamate. When a cell is damaged, it leaks this enzyme into the blood. Therefore the presence of these enzymes in normal indicates liver damage like bile duct obstruction, intra heptic cholestasis or infiltrate diseases of the liver. It was found that the treatment of HCC using *A. ilicifolius* leaf extract exhibited significant change in the levels of AST, Alkaline phosphatise and ALP in HepG2 cell line induced HCC. The increase in the levels of AST, ALT, ALP during the treatment indicate the significant restoration of all the three biochemical parameters towards their control values.

Urea is one of the main antioxidant present in the body and Hepatocellular carcinoma causes elevated levels of urea[17]. The extract reflects the anti oxidant potential as it reduced the oxidative stress and reduced the level of urea in the blood. Hepatocellular carcinoma causes disturbance in the renal function, so the blood creatinine level is elevated[11]. Extract treated group exhibited reduction in serum creatinine level that indicates that extract may exert its effect on renal function.

The presence of various biochemical compounds in *A. ilicifolius* leaves might protect the release of toxic substances. Hepatocellular carcinoma is reported to be

Table 2: Effect of *A. ilicifolius* extract on Hepatic profile in HepG2 cell line induced HCC in mice.

Grouping	AST(U/L)	ALT(U/L)	ALP(U/L)
Normal	52±3.8	17.3±0.73	108.5±7.25
HCC control	96.2±1.4	71.2±1.60	305.5±6.15
HCC+Standard drug	64.2±0.818	31.8±2.21	155.7±6.06
HCC+Plant Extract	84.3±1.86 **	41.6±2.15 **	224.9±6.3 **

**P < 0.01 compared with control.

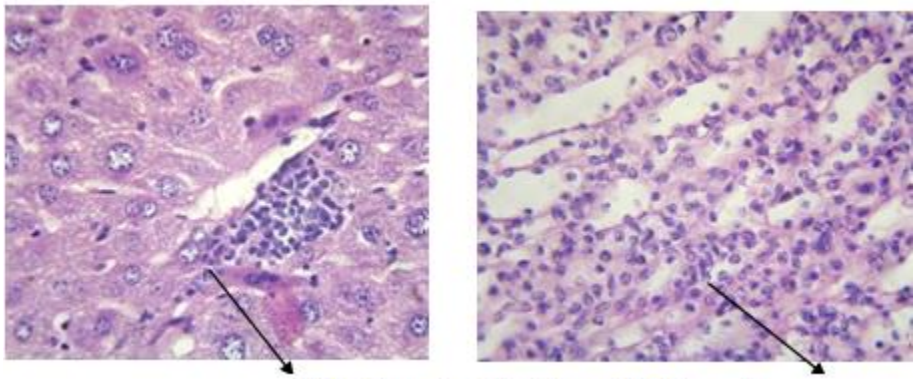

Figure 5: Group 3 HepG2+Standard drug.

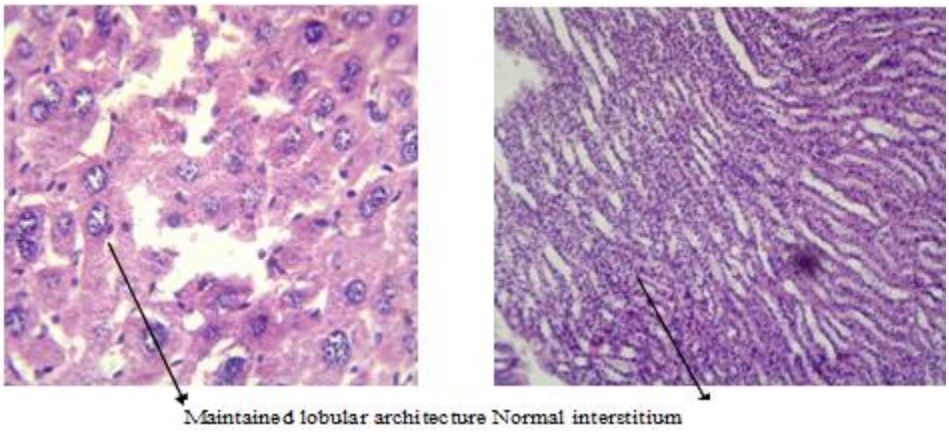

Figure 6: Group-4 HepG2+Plant Extract.

Table 3: Effect of *A. ilicifolius* extract on renal Profile in HepG2 cell line induced HCC in mice.

Grouping	Urea(u/l)	Creatinine(u/l)
Normal	32.5±1.81	0.53±0.165
HepG2 control	69.4±1.34	2.52±0.121
HepG2+Standard drug	56.3±1.70	1.04±0.17
HepG2+Plant Extract	64.3±1.20**	1.36±0.155**

**P < 0.01 compared with control.

associated with the decreased Hb content because of reduced erythrocyte deformability. The reduced deformability leads to a shortening of erythrocyte life span[18]. The decreased Hb level is frequently seen in hepatocellular carcinoma 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. Hepatocellular carcinoma generally results in the accumulation of leukocytes. In the present study the migration of leukocytes into the inflammed area is significantly suppressed by *A. ilicifolius* [19]. Histopathology in mice reveals that the increase in binucleated cells may be due to lipolytic characteristics of Parathinon that alter the permeability of cell membranes of the hepatocytes[20]. This finding indicates the significance of the *A. ilicifolius* plant as a potential resource for the discovery of novel leads for effective and safe anticancer agents.

CONCLUSION

The ethanolic extract of *A.ilicifolius* significantly inhibited tumor in HepG2 cell line induced hepatocellular carcinoma in mice. This activity involves restoration of biochemical and Histopathology parameters, reduction in tumor volume and increased lifespan of the animals. These results suggest that *A.ilicifolius* might be a good choice for the treatment of cancer. Drug seekers can use as a tumour potential agent of anticancer chemotherapy.

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