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RESEARCH ARTICLE

Evaluating the Antioxidant and Anticancer Activity of *Saccharum spontaneum* Linn Flower Extract in Hepatocellular Carcinoma: An *In Vitro* and *In Vivo* Study

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Abstract

Background: Advanced Hepatocellular Carcinoma (HCC) is a devastating disease with a poor survival rate, emphasising the urgent need for novel and effective therapeutic agents. *Saccharumspontaneum* Linn *(S. spon)*, a member of the *Poaceae* family, has garnered attention due to its diverse pharmacological benefits, including antibacterial, antioxidant and anti-inflammatory properties. **Aim:** This study aims to investigate the phytochemical composition of *S. spon* flower extract and evaluate its antioxidant and anticancer potential against HCC. **Methods:** The antioxidant activity of the Methanolic Extract (ME) of *S. spon* flowers was assessed using DPPH and Ferric Reducing Antioxidant Power (FRAP) assays. The anticancer efficacy was evaluated using MTT assay on HepG2 cell lines. An *in vivo* study was also conducted using a diethyl nitrosamine-induced hepatocellular carcinoma animal model. **Results:** The results showed significant antioxidant activity in the DPPH and FRAP assays. The MTT assay revealed a dose- and time-dependent decrease in cell viability, with an IC50 value of 25.5 µM/mL. Furthermore, the ME *S. spon* exhibited notable cytotoxic effects and mitigated liver injury in the animal model, as evidenced by decreased liver enzyme levels ($p < 0.05$). **Conclusion:** This study demonstrates that *S. spon* possesses significant antitumor efficacy and pharmacological potential, making it a promising candidate for the treatment of hepatic cancer. The findings suggest that *S. spon* inhibits cancer cell growth and induces programmed cell death, warranting further investigation for its therapeutic applications.

Keywords: Diethyl Nitrosamine, Hepatocellular Carcinoma, HepG2cell Lines, *Saccharum spontaneum* Linn, Trans Arterial Chemoembolisation

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1. Introduction

HCC is the most prevalent kind of initial liver cancer and ranks as the third highest underlying factor of cancer-related deaths worldwide[1]. Common risk factors include aflatoxin contact, excessive consumption of alcohol, chronic infection with hepatitis B and C viruses, Non-alcoholic fatty liver disease, obesity, diabetes and metabolic syndrome. These factors, whether individually or in combination, gradually harm the liver, leading to genetic as well as epigenetic modifications that affect many microenvironmental conditions[2]. The treatment strategy for HCC is determined by the stage of the illness. Patients diagnosed with early-phase HCC are eligible for excision or aggressive therapies which include cryosurgery, liver replacement therapy, or local ablation using Percutaneous Ethanol Ablation (PEI) or Radio Frequency Ablation (RFA). These treatments have shown 5-year survival rates varying from 41% to 74%[3,4]. Trans Arterial Chemoembolisation (TACE) is beneficial for people with mid-stage HCC. Nevertheless, those diagnosed with progressive HCC have a very unfavourable outlook, with a mere 6.5 to 10.7 months of overall survival after sorafenib therapy[5,6]. Hence, it is crucial to prioritise the creation of potent new medicines that have lower toxicity to enhance the chances of survival for individuals with advanced HCC.

Currently, there are several methods for the treatment of cancer. Nevertheless, cancer cells possess the capacity to withstand traditional treatments[7]. Additional investigation is necessary to produce efficacious cancer therapies due to the rising incidence of therapeutic resistance in cancer cells against antineoplastic therapy[8]. Consequently, several researchers have endeavoured to enhance the antitumor efficacy of antineoplastic therapy by integrating it with certain organic substances[9,10]. *S. spon* is a traditional herb, sometimes referred to as Kasa, wild cane and wild sugar cane, that belongs to the *Poaceae* family[11]. The distribution of this species is extensive in India, particularly in the states of Andhra Pradesh and Tamil Nadu, where it is often found along the riverbanks[11]. This plant has a diverse range of chemical compounds including quinone, alkaloids, saponins, tannins, carbohydrates, proteins, coumarin, phenol, steroids, terpenoids and glycosides[12].

This research aimed to estimate the phytochemical components found in the ME *S. spon* flowers. Furthermore, the ability of the plant extract to inhibit the growth of HepG2 cells in laboratory as well as animal studies is examined.

2. Materials and Methods

2.1 Collection and Preparation of Plant Materials

Flowers of *S. spon* were collected from Ammapettai in Chengalpet Taluk. The collected floral plant part was identified by a botanist. The freshly collected plant material was subsequently fragmented and desiccated in a shaded environment, followed by pulverisation into a fine powder using a mechanical grinder. The resulting powder was extracted using the maceration process with methanol solvent.

2.2 Material Characterisation

The Fourier Transformation Infrared (FTIR) spectroscopy was conducted using a PerkinElmer 400 FT-IR/FIR spectrometer from the United States. The spectroscopy covered between 400cm^{-1} to 4000cm^{-1}. The spectral resolution was maintained at 0.4cm^{-1} [13].

2.3 Antioxidant Assay

2.3.1 DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging capability of *ME S. spon* was assessed using a conventional approach with minor adjustments[14]. In summary, 100 microliters of *ME S. spon* were diluted serially on a 96-well plate, with concentrations ranging from 6.75 to 100 mg/ml. Subsequently, a solution of DPPH with a concentration of 0.2M was added to each well, with a volume of 100ml. Subsequently, the constructed well plate was placed in a dark environment and incubated for 30 minutes. The Abs was analysed using a spectrophotometer at a wavelength of 517nm. The experiment was conducted in triplicate using a ME *S. spon*. DPPH and ascorbic acid were used as negative and positive controls, respectively, with methanol as the blank in the DPPH scavenging activity assay. The

calculation of RSA was performed using the following method:

$$Free\ Radical\ Scavening\ Activity(\%) = \left(\frac{Control(Abs) - Test\ Sample(Abs)}{Control(Abs)}\right) * 100$$

2.4 Qualitative Phytochemical Analysis

The extract underwent testing to determine the occurrence of bioactive chemicals using established standard procedures[15].

2.5 *In Vitro* Studies

2.5.1 Culturing of HepG2 Cell Line

The HepG2 cells ATCC-HB-8065 were procured from and grown in a 5% National Centre for Cell Science (NCCS), Pune and the cells were incubated in a CO_2 incubator at 37°C using Dulbecco's Modified Eagle Medium (DMEM). After reaching 80% confluence, the cells were sub-cultured using a Trypsin-Ethylenediaminetetraacetic Acid (EDTA) solution and used for further investigations.

2.5.2 FRAP Assay

The antioxidant capacity of all nanoparticles synthesised by green methods was assessed using the FRAP assay, following the approach of Benzie and Strain with minor adjustments[16]. HepG2 cells were placed onto a 96-well plate and incubated for 24 hrs. Following incubation, the cells were exposed to various doses of ME *S. spon* (control, std. drug (vitamin C), 10, 25 and 50 µg/ml). 100µl of chromogenic substrate was applied to each well and incubated for 10mins. in darkness at ambient temperature. Following the reaction, a varying range of blue hues was detected. Ultimately, the measurement of absorbance was conducted at a wavelength of 560nm utilising an Enzyme-Linked Immunosorbent Assay (ELISA) microplate reader. The calculation of Radical Scavenging Activity (RSA) was performed using the following method:

$$FRAP\ Scavening\ Activity(\%) = \left(\frac{Control(Abs) - Test\ Sample(Abs)}{Control(Abs)}\right) * 100$$

2.5.3 Cytotoxicity Assay

The ME *S. spon* flowers were evaluated for their cytotoxic effects on HepG2 cells utilising the MTT test[17]. HepG2 cells were placed onto a 96-well plate and incubated for 24 hours. The cells were then incubated for an additional 24 hours in a 5% CO_2 incubator at 37°C. Following incubation, the cells were exposed to various doses of ME *S. spon* (control, 5-FU, 10, 25, and 50 µg/ml). The cells were subsequently incubated for a further 24 hours in a 5% CO_2 incubator at 37°C. Afterwards, the cells were washed with Phosphate Buffered Saline (PBS) and exposed to serum-free media formulated with 10µl of MTT (0.5/ml) for 4 hours in a dark environment. The insoluble formazan crystals were dissolved using a 100µl solution of Dimethyl Sulfoxide (DMSO). The absorbance of the corresponding was then meticulously analysed spectroscopically at a wavelength of 545nm using a microplate reader. The calculation of cell viability percentage was performed using the following method:

$$Cell\ Viability(\%) = \left(\frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells}\right) * 100$$

2.6 *In Vivo* Studies

2.6.1 Animal Model

The research was conducted after receiving animal ethical clearance from the Institutional Animal Ethical Committee (IAEC 3/Proposal:93/A. Lr:65/Dt:22.08.2022) of Chettinad Academy of Research and Education. 30 male Wistar albino rats were acquired from Mass Biotech-Lab Animal Research, located in Chengalpattu. The subjects were housed in hygienic polypropylene enclosures, maintained at a temperature range of 23-25 ºC and a humidity level of 50-60%. The subjects were exposed to a 12-hour light/dark cycle, alternating between periods of illumination and darkness. Their diet consisted of unlimited access to water and pellets. The animals underwent a one-week quarantine period to ensure their physiological, psychological and dietary stability. The body weight of individual rats was assessed before commencing the experiment, once per week during the duration of the trial, and after the experiment. The feed weight was also determined to measure their dietary rate before, throughout and following the experiment.

2.6.2 Hepatocellular Carcinoma Induction

The chemical Diethyl Nitrosamine (DEN) was administered to the rats via the intra-peritoneal (i.p) route.

2.6.3 Grouping of the Animals

The 30 animals were distributed among 5 experimental groups, with six animals respectively. The experiment consisted of five groups: group 1, which was left blank; Group 2, which was assigned as the negative control and injected DEN (30 mg/kg b. wt); Group 3, which was assigned as the positive control and injected DEN (30 mg/kg b. wt) along with silymarin (30 mg/kg b. wt); Group 4, which injected DEN (30 mg/kg b. wt) along with plant extract (200 mg/kg b. wt); and Group 5, which injected DEN (30 mg/kg b. wt) along with plant extract (400 mg/kg b. wt). In the conclusion of this study, blood samples were taken and analysed for haematological parameters, Liver Function Tests (LFT) and alpha-fetoprotein levels. Subsequently, animals were subjected to sacrificial procedures, and samples of the liver were collected for histological analysis[18].

3. Statistical Analysis

The data was statistically evaluated using GraphPad Prism. The study was conducted triplicate for each test, and the results were evaluated using the formula for the average value plus or minus the standard deviation. The data was analysed employing One-way ANOVA, and Bonferroni's multiple comparison test ($p < 0.05$) was used to detect any significant differences across the samples.

4. Results

4.1 Characterisation

4.1.1 FTIR

The FTIR approach was utilised to get information about the emission spectrum by analysing the vibrational mode characteristics of the plant extract. The FTIR spectrum analysis reveals distinct peak locations for the samples, as seen in Figure 1. The band at 3444 cm^{-1} detects O-H stretching occurrence of class alcohol, band at 1645 cm^{-1} detects C=N stretching occurrence of class imine/oxime, band at 1390 cm^{-1} detects S=O stretching occurrence of class sulfate, band at 1040 cm^{-1} detects CO-O-CO stretching occurrence of class anhydride and band at 600 cm^{-1} detects C-Br stretching occurrence of class halo compound[19].

4.2 Antioxidant Assay

4.2.1 DPPH Free Radical Scavenging Assay

The DPPH free radical inhibition activity of several substances at different doses is shown in Figure 2. The

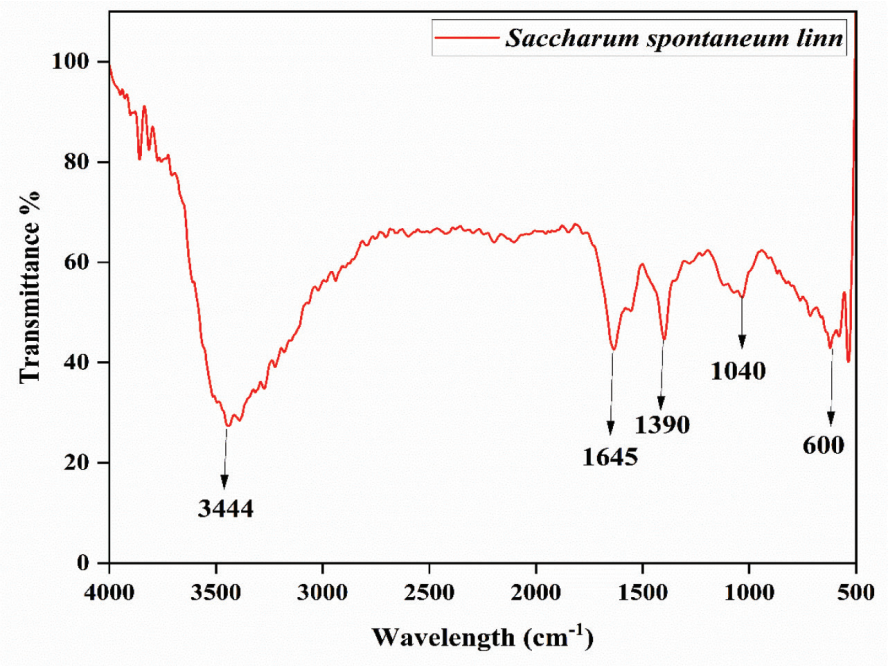


Figure 1. FTIR spectrum of ME *S. spon*.

inhibitory effect observed with these compounds was much greater than that of the other compounds and, to a certain degree, was equivalent to the standard, namely ascorbic acid. Figure 2 depicts the influence of various concentrations of plant extract on the antioxidant activity of DPPH radicals. The results of our investigation indicated that the plant extract exhibited comparable antioxidant activity to ascorbic acid. The antioxidant activities in the DPPH test rely on the plant extract's capacity to transfer electrons to the unpaired electron in the DPPH sol. As a result, the strength of the n → π* transition at 517nm decreases, creating a change in colour from deep purple to light yellow[20]. The observed change in colour indicates the existence of antioxidant action.

4.3 Qualitative Phytochemical Analysis

The phytochemical properties of the ME *S. spon* are summarised in Table 1. The analysis showed the existence of pharmacologically active substances in the ME *S. spon*. The table reveals the occurrence of carbohydrates, phenols and tannins, flavonoids and saponins of ME *S. spon* flowers.

4.4 *In Vitro* Studies

4.4.1 FRAP Assay

During the FRAP experiment, the inclusion of TPTZ allows for the measurement of an antioxidant's ability to convert Fe^{3+} to Fe^{2+}. This conversion produces a distinct blue Fe^{2+}-TPTZ complex, which can be detected at its maximum absorption wavelength of 59nm. The FRAP measurements were expressed as micromolar equivalents of Fe (III) reduction per gramme of dry sample weight. The findings demonstrated that the ME *S. spon* flowers exhibited a notably greater capacity for reduction at the highest concentration (Figure 3). These values were considerably minimised ($p<0.05$) than the control group.

4.4.2 Cytotoxicity Assay

The cytotoxicity of ME *S. spon* on HepG2 cells was assessed using the MTT test. Figure 4 exhibited the cytotoxicity of ME *S. spon* in HepG2 cell lines, which

Table 1. Phytochemical screening from ME *S. spon*

Name of the Test	Methanolic Extract
Fehling	+
Iodine	+
Phenol and Tannin	+
Shinoda	+
Alkaline	+
Saponin	+
Liberman	+
Salkowski	+
Keller-Kilani	+
Steroid	+
Terpenoid	+
Alkaloid	+

Where '+' represents the occurrence and '-' represents the absence.

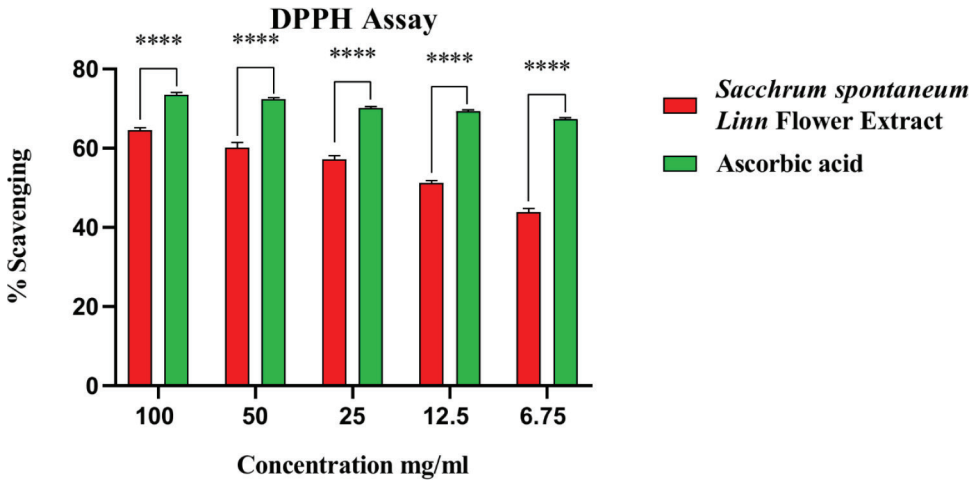


Figure 2. DPPH free radical inhibition at various concentrations. Values are presented as mean ± SD (n=3). ****p<0.0001.

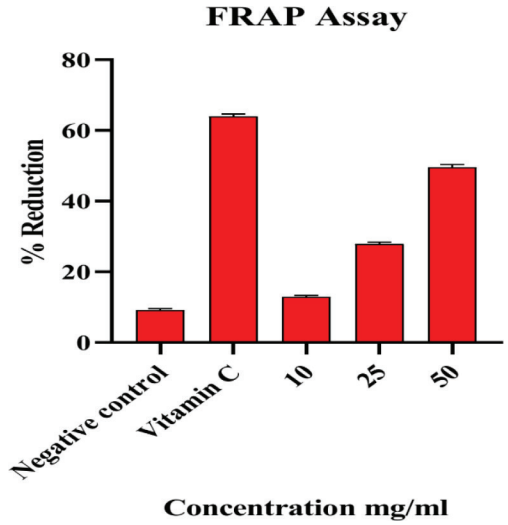


Figure 3. FRAP reduction percentage at various concentrations of *Saccharum spontaneum* Linn against Hep G2 cell lines.

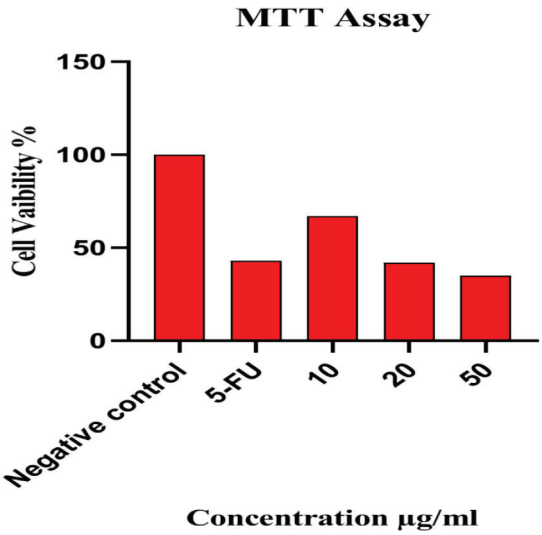


Figure 4. Cytotoxicity efficiencies of ME *S. spon* against HepG2 cell lines.

was dependent on the dosage (10, 25, 50 µg/ml). The observed morphological changes, including cellular rounding and reduction in cell volume, were associated with toxicity. The cell viability percentages of floral *ME S. spon* at concentrations of 10, 25 and 50 µg/ml were determined to be 67±1.89, 42±2.31, and 35±2.24, respectively. In comparison, the cell viability % of 5-FU (5mmol) was found to be 43 ± 2.43. These values were considerably lower ($p<0.05$) than the control group. The IC50 value of ME *S. spon* on HepG2 human liver cancer cells, 48 hours after stimulation, was determined to be 25.543µg/ml. The precise signalling mechanisms, such as the mitochondrial or death receptor pathways, result in the initiation of apoptosis[21,22]. Furthermore, the examination of alterations in gene expression associated with programmed cell death, the body's defence against oxidative stress, and the ability of cells to survive, including the control of genes that promote or inhibit apoptosis, yields vital data[23-25]. Moreover, it is crucial to analyse the activation or inhibition of proteins, such as caspases and kinases and to study how the extract affects the levels of Reactive Oxygen Species (ROS), which may trigger apoptosis or activate antioxidant defences[26-28].

4.5 *In Vivo* Studies

4.5.1 Effect on Body Weight

The final body mass index of the control group was (331.03 ± 62.81) g, whereas group II, after receiving DEN, exhibited a substantial drop in body weight of (114.83 ± 126.68) g. In groups III, IV and V, the administration of silymarin (30mg/kg b.wt) and ME *S. spon* (200, 400mg/kg b.wt) together with DEN induction resulted in a significant increase in weight (202.50 ± 167.68, 150.50 ± 111.66, 178.33 ± 138.52)g, respectively, compared to the animals in group II that were only treated with DEN. Table 2 presents the impact of the ME *S. spon* on body weight in a liver cancer model generated by DEN.

Table 2. Effects of ME *S. spon* on body weight in DEN-induced liver cancer model

Groups	Baseline 0 Mean±SD	Week 10 (final weight) Mean ± SD	P-value
I	135.17±41.02	331.03± 62.81	.000
II	120.00± 26.87	114.83± 126.68*	.018
III	121.50± 26.74	202.50± 167.68@	.008
IV	125.00±42.53	150.50± 111.66@	.529
V	130.83±17.57	178.33±138.52@	.000

Values are Mean ± SEM(n=6), *p≤0.05 versus the control group I. @p≤0.05 versus the group II (DEN treated), where GIII, GIV and GV compared with GII (positive control) is significantly increased ($p<0.05$).

4.5.2 Effect on Hematological Parameters

Table 3 shows that in Group II (negative control), there was a statistically significant ($p<0.05$) drop in Haemoglobin (HB), White Blood Cells (WBC), platelets and Red Blood Cells (RBC) compared to the standard control group. Nevertheless, the groups treated

with ME *S. spon* and silymarin exhibited significant enhancements in haematological parameters in a dose-dependent manner (at doses of 200mg/kg and 400mg/kg body weight), in comparison to the illness group (G II).

4.5.3 Effect on Bilirubin and Albumin Levels

Figure 5 displays the amounts of total bilirubin and albumin in both control and experimental animals. The animals exposed to DEN showed a significant reduction in albumin levels compared to the control group. Remarkably, the administration of ME *S. spon* to rats induced with DEN resulted in a noteworthy elevation in both total bilirubin and albumin levels, as compared to animals induced with DEN alone.

4.5.4 Effect on Tumor Marker Enzymes

4.5.4.1 Effect on Alanine Transaminase (ALT)

Administering DEN (30mg/kg) intraperitoneally once per week for ten consecutive weeks in rats resulted in a substantial rise in ALT levels (control: 32.8± 4.4 U/L; DEN: 136.8 ± 12.3 U/L)(Figure 6). However when rats were given an orally administered ME *S. spon* once a week for ten consecutive weeks in combination with DEN, there was a significant reduce in ALT levels (ME *S. spon* 200mg/kg: 69.2 ± 7.6 U/L; ME *S. spon* 400mg: 102.8 ± 11.2 U/L). Additionally, when group III injected a combination of DEN and a standard drug, a significant decrease in ALT levels also occurred (silymarin: 34.7 ± 6.9 U/L).

4.5.4.2 Effect on Aspartate Aminotransferase (AST)

This resulted in a significant rise in AST levels (control: 185.9 ± 12.1 U/L; DEN: 460.0 ± 51.3 U/L) (Figure 6). No significant elevation in AST levels was detected when a combination of ME *S. spon* and DEN was administered. The AST levels for the DEN + Sspon (200mg) group were 244 ± 8.5 U/L, while for the DEN + ME *S. spon* (400mg) group they were 214 ± 12.1 U/L. Similarly,

Table 3. Effects of ME *S. spon* (200mg/kg, 400mg/kg) on haematological parameters in DEN-induced animal model

Groups	Mean±SD				
	HTC(%)	Hb(g/L)	WBC (cells/L)	RBC (cells/L)	PLT (lakhs/µL)
I	56 ±0.51	17.9±0.51	11.3±0.16	8.3±0.90	6.04±0.13
II	37±1.13*	12.7±0.43*	18.5±0.18*	5.4±0.59*	4.08±0.65*
III	55±0.71@	17.2±0.67@	12.4±1.67@	7.7±0.25@	6.44±0.10@
IV	50±1.25@	15.5±1.23@	13.1±1.08@	6.8±0.26@	5.26±0.25@
V	48±2.52@	16.1±0.46@	12.9±0.82@	5.5±0.30@	5.07±0.08@

Values are Mean ± SEM(n=6),*Significantly reduced($p<0.05$) compared to the control group, @significantly elevated $p<0.05$ compared to positive control.

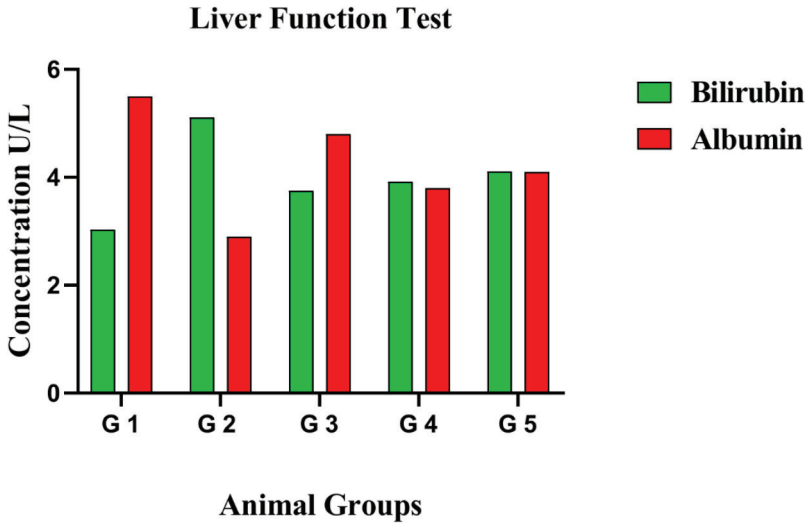


Figure 5. Effect of ME *S. spon* on bilirubin and albuminin of DEN-induced animal model.

the standard medication group (DEN + silymarin) had AST levels of 210 ± 8.5 U/L.

4.5.4.3 Effect on Lactate Dehydrogenase (LDH)

The LDH levels in rats that were administered DEN intraperitoneally (30 mg/kg body wt. once a week for ten weeks) showed a considerable rise (control: 361.2 ± 22.6 U/L; DEN: 1678.6 ± 50.9 U/L) (Figure 6). LDH levels did not increase when DEN was delivered in combination with ME *S. spon* (DEN + ME *S. spon* (200mg): 989.3 ± 148.6U/L); DEN + ME *S. spon* (400mg): 1004.6 ± 86.5U/L) and also in the usual medication group (DEN + silymarin: 633.8 ± 187.4U/L).

4.5.4.4 Effect on Alpha-Fetoprotein (AFP)

The levels of the tumour marker AFP significantly increased due to the administration of DEN (30mg/kg body weight in rats) once a week for ten consecutive weeks (Figure 7). The control group had an average AFP level of 13.22 ± 0.6 ng/ml, whereas the DEN group had an average AFP level of 53.2 ± 2.4 ng/ml. When ME

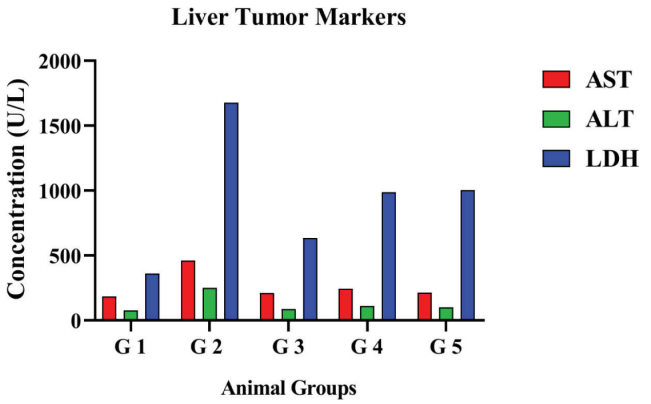


Figure 6. Effect of ME *S. spon* on liver enzymes in DEN-induced animal model.

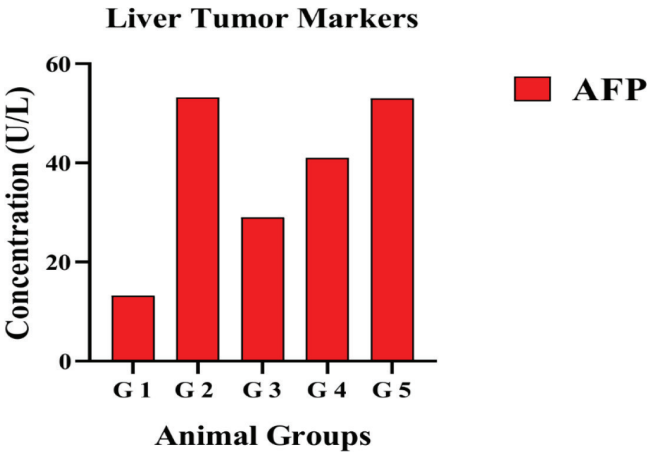


Figure 7. Effect of ME *S. spon* on AFP in DEN-induced animal model.

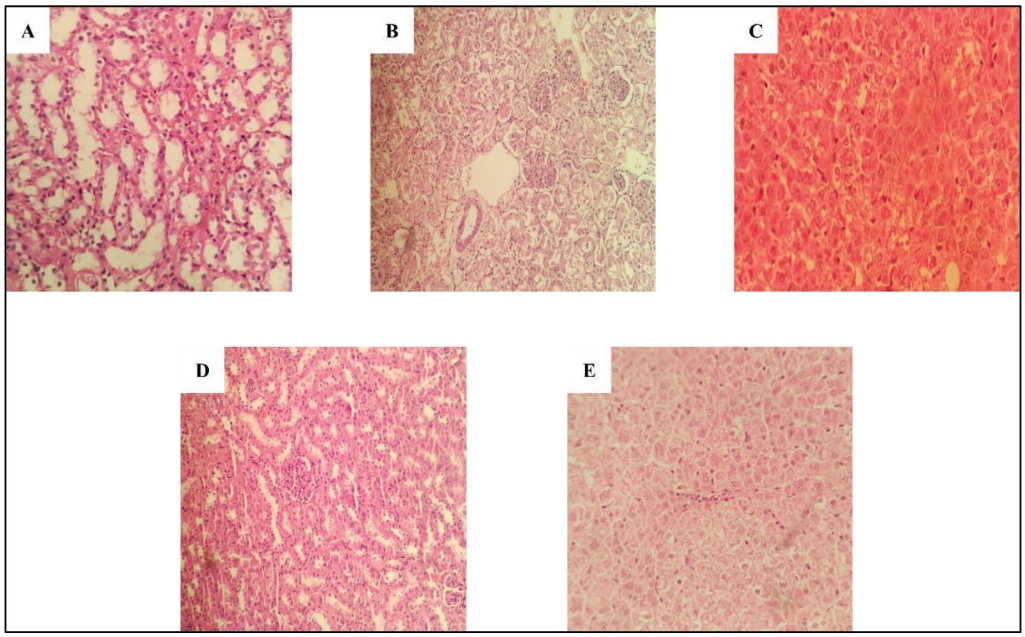


Figure 8. Microscopic images of H and E staining section of the liver. **(A).** Control (untreated) group, **(B).** DEN induced group, **(C).** DEN with the standard drug, **(D).** DEN with ME *S. spon* 200 mg/kg b.wt. and **(E).** DEN with ME *S. spon* 400 mg/kg b. wt.

S. spon were administered together with DEN, there was no significant rise in AFP levels. The AFP levels for DEN + ME *S. spon* (200mg) were 41.1 ± 5.4 ng/ml, for DEN + ME *S. spon* (400mg) were 35 ± 5.6 ng/ml, and for DEN + Silymarin were 31 ± 6.9 ng/ml.

4.5.5 Histopathological Examinations

The microscopic images showing the HE-stained sections of the liver from control rats showed distinct, symmetrical, mono-nucleated liver cells with a precise outline and intimate proximity to adjacent cells (Figure 8). Sections of the liver exposed to DEN exhibited an increased occurrence of abnormal cells and fragmented nuclei. Nevertheless, liver sections from rats subjected to DEN + ME *S. spon* and DEN + silymarin seemed to counteract the structural alterations caused by DEN exposure.

5. Discussion

HCC is a widespread and lethal variant of liver cancer that is seen all over the globe[29]. Although there have been improvements in therapy, the task of controlling hepatocellular carcinoma remains difficult owing to a range of variables. The objective of treatment is to eliminate cancer cells and alleviate the burdens associated with the tumour. Plant chemicals with apoptotic properties have shown potential in the prevention and therapy of cancer. Plant polyphenols have a notable ability to trigger apoptosis in cancer cells[30]. Moreover, antioxidants with anti-inflammatory characteristics have the potential to fight against cancer, considering the association between oxidative stress, inflammation and the formation of tumours.

Thigalam[31] estimates the antioxidant, antibacterial and anticancer of *Saccharum spontaneum* Linn extract. The work found that the plant extract shows a prominent antioxidant, antibacterial and anticancer activity.

The findings demonstrated the notable cytotoxic properties of the ME *S. spon* against liver cancer cells (HepG2) by the use of MTT and FRAP tests. More precisely, the MTT test revealed that ME *S. spon* exhibits anticancer properties by reducing the viability of cancer cells in a way that is dependent on the dosage. The ME *S. spon* flowers, specifically at a concentration of 50 µg/mL, demonstrated significant dose-dependent cytotoxicity in HepG2 cells as compared to the control. Thus, these substances have the potential to interfere with the growth of cancer cells, induce programmed cell death, or hinder the formation of new blood vessels. In addition, the FRAP test was performed to evaluate the antioxidant activity of the ME *S. spon*. The findings demonstrated a significant antioxidant impact of ME *S. spon* flowers on HepG2 cells, as compared to the control.

During the *in vivo* experiment, liver cancer was produced in groups II-V by giving DEN. The findings indicated that group II (G: II), which injected just DEN treatment, had a substantial decline in weight over time. Nevertheless, the groups who were administered the ME *S. spon* (G: IV, V) had a substantial elevate in weight in versus to the control group. Biochemical marker enzymes are often used to test for liver disorders and evaluate the effectiveness of therapy. The results of this research indicate that treatment with DEN in rats significantly affects liver function, resulting in increased levels of enzymes such as aspartate transaminases, alanine transaminases and lactate dehydrogenases in the bloodstream, as versus to the control group. On the other hand, rats who injected ME*S. spon* (G: IV, V) showed a notable enhancement in liver functions as compared to the group of rats treated with DEN. Animals that were given DEN showed increased levels of AFP, which is a sign of HCC. Nevertheless, the treated groups of ME *S. spon* exhibited a significant decrease in AFP levels, suggesting possible anti-tumor effects against hepatocellular carcinoma (HCC). Furthermore, the histopathological investigation demonstrated notable hepatic injury in rats treated with DEN. However, the groups treated with silymarin and ME *S. spon* flowers showed decreased liver damage accompanied by little fibrosis.

The haematological parameters and liver marker enzymes in rats treated with silymarin and ME *S. spon* flowers showed significant improvements compared to the group treated with DEN. Rats that were given doses of 200 and 400 mg/kg b.wt. of ME *S. spon* exhibited substantial improvements in each liver marker enzyme, as versus to the group treated with DEN. The main factor for this was the occurrence of phytochemicals in these extracts, particularly flavonoids, phenols, and alkaloids. These compounds likely acted together to eliminate the free radicals generated by the injection of

DEN. Therefore, ME *S. spon* flowers400mg/kg/b.wt has great potential as a medication for avoiding liver injury and dysfunction, while also protecting liver tissue from oxidative harm.

5. Conclusion

The cytotoxicity and apoptotic effects of extracts from the floral parts of *Saccharum spontaneum* Linn were evaluated for the first time on the HepG2 cell line. The ME *S. spon* flowers have inhibitory activities against hepatocellular cancer cells, indicating its potential as a therapeutic agent. The cytotoxicity found against HepG2 cells indicates that the ME *S. spon* flowers may include chemicals with the ability to specifically target and inhibit the proliferation of cancer cells. This makes it a promising candidate for future investigation. Among the extracts tested on rats, the ME *S. spon* flowers at a dosage of 400mg/kg/b.wt showed the highest levels of anticancer and antioxidant activities.

This finding suggests that this plant product has tremendous potential as a source for future study. Further research using other *in vitro* models for various types of cancer, as well as relevant preclinical investigations, to determine the specific chemicals responsible for the cytotoxic effects and understand their mechanism of action is required. This work conclusively demonstrates the strong evidence for the anticancer activities of ME *S. spon* flowers and highlights its potential as a significant resource in the battle against cancer. The objective of this study is to fully harness the therapeutic capabilities of ME *S. spon* flowers and facilitate the creation of precise and efficient anticancer therapies via ongoing research and discovery.

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