

## SCREENING OF ANTI-COLON CANCER ACTIVITY BY USING HYDROALCOHOLIC EXTRACT OF *BORASSUS FABILIFERS* HOOT ON SWISS ALBINO MICE

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### ABSTRACT

**Objectives:** This study addresses the escalating global incidence of colon cancer, recognized as the most prevalent cancer type. With approximately 4.6% of men and 4.2% of women diagnosed during their lifetime, the urgency to explore effective treatments becomes paramount, given the annual occurrence of 1.23 million new cases worldwide. The primary objective is to investigate the impact of the hydroalcoholic extract of *Borassus flabellifer* on altered hematological and biochemical parameters induced by azoxymethane (AOM)/dextran sulfate sodium (DSS), identifying potential hepatoprotective effects.

**Methods:** This research utilized 30 mice, with 24 receiving AOM injections and 6 serving as untreated healthy controls. The AOM-injected mice were further subdivided into groups, and treatment with *B. flabellifer* extract commenced on the 3<sup>rd</sup> day and spanned 70 days. DSS cycles were administered, and hematological and biochemical parameters were assessed. At the end of the experiment, mice were sacrificed, and the colorectal region was preserved for analysis.

**Results:** Results elucidate the AOM/DSS-induced alterations, including increased serum levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, white blood cell, platelet count, and total protein, indicative of hepatic damage. Conversely, the hydroalcoholic extract significantly reduced these parameters, aligning with previous studies showcasing the plant's anti-cancer activity. These findings highlight the pharmacological potential of *B. flabellifer* in mitigating colon cancer symptoms.

**Conclusion:** This research underscores the significance of *B. flabellifer* in countering the rise of colon cancer. The hydroalcoholic extract exhibits promising results in ameliorating AOM/DSS-induced alterations, providing a foundation for further exploration of its therapeutic potential. The study contributes valuable insights into the pharmacological properties of *B. flabellifer* and sets the stage for potential applications in cancer treatment.

**Keywords:** Colorectal cancer, *Borassus flabellifer*, Hydro-alcoholic extract, Cancer therapies.

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### INTRODUCTION

Colorectal cancer (CRC) stands as the third most prevalent cancer globally, marked by uncontrolled cell growth and proliferation. According to the American Cancer Society, CRC affects approximately one million individuals worldwide annually. In 2019 alone, there were 101,420 reported cases of colon cancer, 40,180 cases of rectal cancer, and 51,020 CRC-related deaths. Notably, CRC incidence is on the rise in developing nations, attributed to shifts in lifestyle patterns toward Westernization, including increased smoking, alcohol consumption, obesity rates, sedentary lifestyles, and higher intake of red meat. As a result, CRC significantly impacts global mortality and morbidity rates [5].

CRC treatments are currently limited, costly, and often accompanied by side effects. The side effects of chemotherapy include bone marrow suppression, hair loss (alopecia), cognitive impairment (chemobrain), sensitivity to cold, peripheral neuropathy (numbness, tingling, blistering of fingers and feet), bleeding, fatigue, etc. As well as changes in bowel habits (diarrhea, constipation, frequent bowel movements), abdominal discomfort (bloating), urinary and genital problems, surgical complications, skin, bladder, and rectal irritation, secondary tumors, and infertility, especially after pelvic radiation exposure [26].

Plants have been utilized for health and medical purposes for numerous generations. Medicinal plants play a pivotal role in the human healthcare system, offering a unique avenue for the development

of modern drugs [4]. The demand for herbal medicines is significant in the developed world of primary healthcare due to their perceived safety, efficacy, and fewer side effects. However, the true medicinal value of over 4,000 plants remains either little-known or unknown to the mainstream population [22]. Herbal medicines have gained prominence due to their perceived sensitivity to unwanted effects compared to synthetic medicines. This has led to an increased demand for herbal resources and a growing awareness of the importance of maintaining the quality and purity of raw materials [6]. The therapeutic properties of various herbal medicines have been acknowledged in various ancient cultures [24]. This recognition highlights the sustained relevance of traditional knowledge in harnessing the healing potential of plants for the benefit of human health.

The Palmyra tree, scientifically known as *Borassus flabellifer*, stands out for its distinctive characteristics and is abundant in therapeutic qualities. A comprehensive exploration of the therapeutic attributes of this plant [33]. There are many countries in South and Southeast Asia that have this remarkable tree, including Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Myanmar, Sri Lanka, Sulawesi, Vietnam etc. Characterized by its nature, the Palmyra tree boasts a lifespan of over 100 years and can reach an impressive height of 30 m (98 feet). Its canopy, adorned with green-bluish leaves, spans approximately 3 m (9.8 feet) across and serves various purposes, including thatching, making mats, baskets, fans, hats, umbrellas, and torches. The base of young leaf stalks is utilized for straining Toddy, a traditional beverage [16,36].

According to existing literature, dietary polyphenolics present in the Palmyra tree, such as phenolic acids and flavonoids, act as beneficial anti-oxidants capable of neutralizing harmful active oxygen species such as  $O_2$ ,  $H_2O_2$ , and  $-OH$  [25]. Additional findings highlight that the fresh pulp of the tree is rich in Vit-A and C, while the fresh sap serves as a good source of Vit B-complex [12]. Moreover, the Palmyra tree contains traces of various minerals, and its low levels of lead, nickel, and arsenic further indicate its safety, being free of hazardous metals. The investigation into the flowers of *B. flabellifer* encompassed the assessment of their analgesic and anti-pyretic effects [19], anti-inflammatory activity, impact on hematological and biochemical parameters [20], and potential immunosuppressant properties [23]. The utilization of pellets derived from *B. flabellifer* Linn. demonstrated a marked reduction in the ability to induce a delayed-type hypersensitivity response [7]. Additionally, the flour obtained from the young shoots of *B. flabellifer* underwent scrutiny for mutagenicity [3], mitogenic activity [10], and neurotoxic effects. The fruit, characterized by a diameter ranging from 4 to 7 inches, is sizeable and fibrous, typically comprising three nut-like portions, each enclosing a seed [31]. The leaves' stems are adorned with thorny edges. The male inflorescence contains spirostane-type steroid saponins, including borassosides and dioscin, along with 20 recognized steroidal glycosides [13,35] and carbohydrates like sucrose [32,34].

Furthermore, it houses a bitter compound called flabelliferins, which falls under the category of steroidal saponins. *B. flabellifer* is known to consist of gums, albuminoids, fats, with the fresh pulp reputedly rich in vit A and C [8]. The fresh sap serves as a valuable source of vit B-complex [15]. Various components of the plant find applications in medicinal contexts, including antihelminthic and diuretic uses [21]. Traditional dishes often incorporate the fruit pulp of *B. flabellifer*, and its sap is employed as a sweetener for diabetic patients [14]. The genus *Borassus*, consisting of six species of fan palms, witnesses the consumption of its fruits either roasted or in their raw state, and the young, jelly-like seeds are also considered edible [28]. Given the pharmacological properties of the plant and the contemporary trend of employing herbal medicine in cancer treatment, this study specifically employed the hydroalcoholic extract of *B. flabellifer* to evaluate its efficacy against colon cancer in albino mice.

## METHODS

### Plant collection and authentication

Fresh plants of the *B. flabellifer* Linn, were collected during January 2020 from the Nellore District of Andhra Pradesh. It was authenticated by G. Prabhakar junior lecturer in botany, T.N.C. Govt. Jr. College, Kovur, SPSR Nellore, voucher specimen number 00637.

### Extraction process

The plants of *B. flabellifer* (young shoot) were shade-dried. The dried plant was ground to get coarse powder and passed through a 40 mesh. A 450 g of crude powder was subjected to a soxhlation process using hydro-alcohol (70:30).

Percentage yield = Weight of dry extract/Weight of dry material  $\times$  100

### Animal housing

The research study was conducted using female, healthy albino mice of the Swiss strain weighing 20–25 g. The mice were obtained from Sri Venkateswara Agencies in Bangalore. After acclimatization, animals were selected randomly and were divided into five groups (n=6). All the experimental protocols were approved by the Institutional Animal Ethical Committee of Vinayaka Mission's College of Pharmacy (CPCSEA NO: 684/PO/Re/S/02/CPCSEA).

### Acute toxicity studies

According to amended Organisation for Economic Cooperation and Development (OECD) guidelines 423, the acute oral toxicity test of the extract was performed using Swiss mice of the female sex weighing between 20 g and 25 g (8 weeks). For 14 days, the treated animals were observed for mortality and general behavior. Till the completion of the

trial, no deaths were noted. The extract was confirmed to be safe up to a level of 2000 mg/kg.

### Experimental protocol

For this investigation, a total of 30 mice were employed. Among them, 24 mice underwent intraperitoneal injection (i.p.) with 7.4 mg/kg of azoxymethane (AOM), while 6 mice were designated as untreated healthy controls. The mice subjected to AOM injection were subsequently divided into four groups, each consisting of 6 mice, as detailed in the following Table 1.

The administration of the extract started on the 3<sup>rd</sup> day and persisted for a duration of 70 days. Subsequently, 1 week after the extract initiation, the drinking water was substituted with a 2.5% dextran sulfate sodium (DSS) solution for 7 days. Following this, the DSS solution was replaced with regular water for the subsequent 2 weeks, marking the completion of DSS cycle-1. Post the conclusion of DSS cycle-1, the drinking water was again replaced with a 2.5% DSS solution for another 7 days, followed by a recovery period of 2 weeks with regular drinking water, constituting DSS cycle-2. This DSS cycle was iterated to accomplish the third and final round. At the culmination of the entire treatment schedule, the mice were humanely sacrificed through cervical dislocation. The colorectal region was then carefully removed and flushed with phosphate buffer. The colon was longitudinally opened to observe tumor features and subsequently preserved in a 10% formalin solution, following established protocols [11,30].

### Biochemical parameters and its methods

Determination of albumin, total protein content, serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) in mice serum

According to the prior study, albumin, total protein content, SGOT, SGPT, and ALP in mice serum were evaluated [2].

### Estimation of tissue anti-oxidant parameter

Large intestine was isolated, then it was immediately processed for antioxidant parameters like reduced glutathione (GSH) (1), Catalase (CAT) (8,9), superoxide dismutase (SOD) [14], and lipid peroxidation (LPO) [18].

### Statistics

All data are expressed as mean  $\pm$  standard error of the mean. One-way analysis of variance followed by Tukey multiple comparison tests were used to determine statistical significance among more than two groups using a computer-based fitting program (Prism, Graph pad 5). The significance level was set at  $p < 0.001$  for all tests.

## RESULTS

The hydroalcoholic extract of shoots of *B. flabellifer* was prepared using soxhelt apparatus, and the percentage yield was found to be 27.44%w/w.

Table 1: Experimental design of grouping for mice

S. No.	Group (n=6)	Treatment
1.	Normal	Vehicle
2.	Disease control	Tumor-bearing mice/AOM/ DSS-induced colorectal cancer
3.	Standard (5-Fluorouracil 20 mg/kg i.p)	Tumor-bearing mice treated with Standard drug (5-Fluorouracil 20 mg/kg i.p)
4.	<i>Borassus flabellifer</i> extract (200 mg/kg)	Tumor-bearing mice treated with hydro-alcoholic extract of <i>Borassus flabellifer</i> @ 200 mg/kg bw
5.	<i>Borassus flabellifer</i> extract (400 mg/kg)	Tumor-bearing mice treated with hydro-alcoholic extract of <i>Borassus flabellifer</i> @ 400 mg/kg bw

AOM: Azoxymethane, DSS: Dextran sulfate sodium

### Acute toxicity

The albino mice were investigated for the acute oral toxicity of the hydroalcoholic extract of *B. flabellifer* following OECD 423 guidelines, and the results of Table 2 indicated that there is no toxicity at the highest dose of the extract which is 2000 mg/kg. The animals showed no death, with no changes in skin texture color or hair fall. There was an indication of changes in the behavior of the mice after administration of the extract. Thus, it was concluded that the extract was safe at 2000 mg/kg and, so 1/4<sup>th</sup> and 1/8<sup>th</sup> doses were considered as testing doses (high and low), respectively.

### Effect of hydroalcoholic extract of *B. flabellifer* shoot on body weight

The effect of the extract of *B. flabellifer* was investigated on the body weight changes of the mice, which showed interesting results. In the normal group, there were random spikes of weight changes over a period of 80 days, as shown in Fig. 1. Whereas the disease group showed slight initial weight gain, which later showed a significant fall. Standard group and the low dose of the hydroalcoholic extract of *Borassus flabellifer* & high dose of hydroalcoholic extract of *Borassus flabellifer* showed a slight increase in body weight which can be supported by the food intake of the mice and lack of exercise.

### Effect of *B. flabellifer* on water and food intake

The effect of *B. flabellifer* on the feed and water intake was shown in Fig. 2 and the results indicated that the normal group exhibited a slight

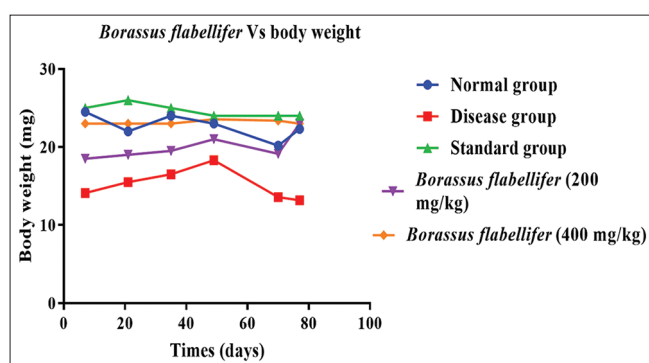


Fig. 1: Effect of *Borassus flabellifer* on the body weight changes

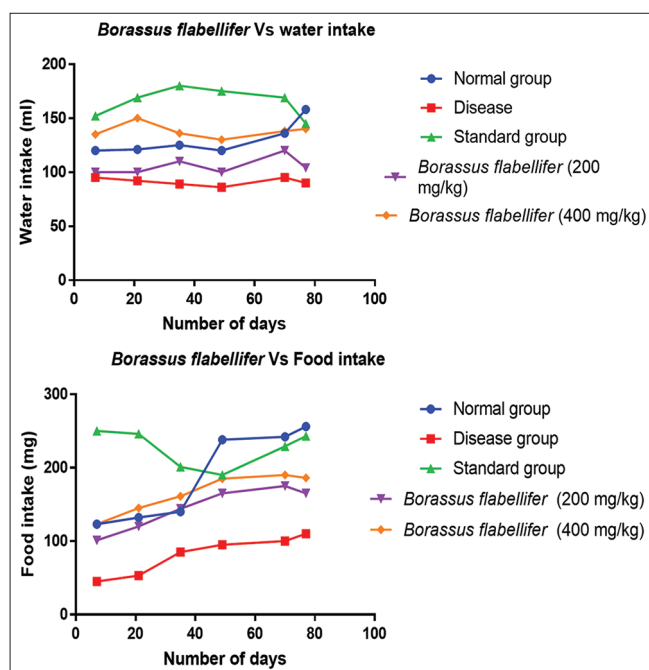


Fig. 2: Effect of *Borassus flabellifer* on the food and water intake of mice

increase in the water intake in the later part of the study from 40 days through 80 days. The standard group and extract-treated groups showed a slight decrease in water intake. The mice in the disease control group showed low water intake from the start of the study, which can be due to the physiological disturbance in the intestine. Contrarily, the feed intake results showed significant changes supporting the weight changes in the previous test. The normal group showed a significant increase in feed intake, whereas other treatment groups showed spikes and dips in the graph. The disease control group also showed increase in the feed intake, but negative effect was observed in the weight gain. Though the feed intake was high, weight loss in the disease group indicates the indication of cancer in the intestine was effective and caused the animal to lose weight.

### Effect of *B. flabellifer* on biochemical and hematological parameters

Table 3 displays the effect of *B. flabellifer* on biochemical and hematological parameters, comparing various groups including the control, disease (AOM/DSS-induced CRC), standard (5-fluorouracil treated), and different doses of *B. flabellifer*-treated groups (200 mg/kg and 400 mg/kg). The results indicate notable changes in several parameters, indicating the potential of *B. flabellifer* in treating CRC. Liver function markers, specifically Alanine transaminase (ALT), Aspartate transaminase (AST), and ALP, displayed significant alterations in the disease group, highlighting elevated levels indicative of degeneration of liver function. However, treatment with *B. flabellifer* at both 200 mg/kg and 400 mg/kg doses demonstrated a substantial reduction in these markers, approaching levels observed in the standard group. For instance, ALT values decreased from  $82.5 \pm 2.849$  in the disease group to  $69.5 \pm 1.91$  (400 mg/kg dose), indicating a potential hepatoprotective effect of *B. flabellifer*.

Biochemical parameters such as total protein content exhibited a significant increase in the disease group ( $8.683 \pm 0.149$ ) compared to the control ( $5.183 \pm 0.197$ ), suggesting systemic inflammation associated with CRC. Treatment groups, particularly at the 400 mg/kg dose, displayed a trend towards normalizing protein levels ( $6.578 \pm 0.3267$ ), indicating a potential stabilizing effect on protein metabolism and overall health improvement. Hematological parameters, including total white blood cell (WBC), platelet count, hemoglobin, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were significantly altered in the disease group. Total WBC increased from  $13.48 \pm 0.2659$  in the control to  $27.84 \pm 0.492$  in the disease group, reflecting an inflammatory response. Treatment with *B. flabellifer*, especially at the 400 mg/kg dose, showed a trend toward normalization of these parameters. Platelet count increased from  $735 \pm 12.42$  in the disease group to  $802.7 \pm 11.51$  (400 mg/kg dose), indicating a potential prevention of thrombocytopenia associated with CRC.

### Effect of *B. flabellifer* on oxidative stress in AOM/DSS – treated mice

Table 4 outlines the effect of *B. flabellifer* on tissue antioxidants, specifically highlighting on parameters such as SOD, Reduced GSH, CAT, and LOP. The results show the significant variations across the different experimental groups, providing insights into the potential antioxidant effects of *B. flabellifer* in the context of AOM/DSS-induced CRC.

SOD is an important enzyme in antioxidant defense, and displayed a significant decrease in the disease group ( $57.79 \pm 1.34$ ) compared to the normal control ( $93.44 \pm 0.233$ ), indicating a lowered antioxidant response. Treatment with extract of *B. flabellifer* at both 200 mg/kg and 400 mg/kg doses showed a substantial increase in SOD levels ( $72.21 \pm 1.16$  and  $80.9 \pm 1.553$ , respectively), reaching the levels observed in the standard group ( $93.13 \pm 0.3427$ ). This suggests a potential enhancement of antioxidant defense mechanisms by *B. flabellifer*. Reduced GSH exhibited a significant reduction in the disease group ( $6.298 \pm 0.057$ ) compared to the control ( $9.742 \pm 0.039$ ). Treatment with *B. flabellifer*, particularly at the 400 mg/kg dose ( $8.27 \pm 0.061$ ), demonstrated a notable restoration of GSH levels, aligning closely with the standard group ( $9.377 \pm 0.28$ ). This indicates a potential role of *B. flabellifer* in preserving cellular antioxidant activity.

**Table 2: Acute toxicity effects of hydro-alcoholic extract of *Borassus flabellifer* at 2000 mg/kg body weight in female Swiss albino mice**

Observations	0 min	30 min	1 h	2 h	4 h	24 h	7 <sup>th</sup> day	14 <sup>th</sup> day
Physical observations								
Body temperature	N	N	N	N	N	N	N	N
Skin color	N	N	N	N	N	N	N	N
Fur color	N	N	N	N	N	N	N	N
Eyes color	N	N	N	N	N	N	N	N
Urine color	N	N	N	N	N	N	N	N
Behavioral parameters								
Mood								
Alertness/exploratory activity	N	N	N	N	N	N	N	N
Eyes opened/closed	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	N	N	N	N
Restlessness	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Irritability	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Reactivity (environment)	N	N	N	N	N	N	N	N
CNS excitation								
Tremors	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Twitches	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Convulsions	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
CNS depression								
Sedation	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N	N	N
Catatonia	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Ataxia	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Autonomic effects								
Defecation	N	N	N	N	N	N	N	N
Lacrimation	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Urination	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Piloerection	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mydriasis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Miosis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Emesis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sensory responses and reflexes								
Sensory responses								
Touch response	N	N	N	N	N	N	N	N
Pain response	N	N	N	N	N	N	N	N
Reflexes								
Pinna	N	N	N	N	N	N	N	N
Corneal	N	N	N	N	N	N	N	N
Somatomotor effects								
Abnormal gait	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Righting reflex	N	N	N	N	N	N	N	N
Body position	N	N	N	N	N	N	N	N
Limb position	N	N	N	N	N	N	N	N
Respiratory effects and death								
Apnea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Dyspnea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Death	NO	NO	NO	NO	NO	NO	NO	NO

N: Normal, CNS: Central nervous system

**Table 3: Effect of *Borassus flabellifer* on biochemical and hematological parameters**

Parameters	Control Group	Disease group (AOM/DSS)	Standard Group (5-FU)	<i>Borassus flabellifer</i> (200 mg/kg)	<i>Borassus flabellifer</i> (400 mg/kg)
ALT	42.5±2.814	82.5±2.849***	45.5±1.708***	69.5±1.91 <sup>aaa</sup>	60.67±1.25 <sup>bbb</sup>
AST	72.33±3.383	123.8±1.86***	74.83±2.738***	92.33±2.092 <sup>aaa</sup>	83.67±2.974 <sup>bbb</sup>
ALP	64.33±3.792	119.2±3.936***	70.17±5.833***	84.5±2.79 <sup>aaa</sup>	76.33±2.813 <sup>bbb</sup>
Total protein	5.183±0.197	8.683±0.149***	5.65±0.149***	7.243±0.223 <sup>aaa</sup>	6.578±0.3267 <sup>bbb</sup>
Total WBC	13.48±0.2659	27.84±0.492***	13.73±0.277***	17.72±0.3333 <sup>aaa</sup>	15.53±0.4062 <sup>bbb</sup>
Platelet count	1075±2.168	513.2±6.8***	1050±11.41***	735±12.42 <sup>aaa</sup>	802.7±11.51 <sup>bbb</sup>
Haemoglobin	17.1±0.0493	12.13±0.291***	16.11±0.341***	13.07±0.1086 <sup>aaa</sup>	14.03±0.228 <sup>bbb</sup>
RBC	7.085±0.111	5.107±0.3825***	7.062±0.028***	6.228±0.2242 <sup>aaa</sup>	6.785±0.0997 <sup>bbb</sup>
MCV	54.5±0.304	46.28±0.284***	54.02±0.4619***	48.01±0.3704 <sup>aaa</sup>	51.46±0.514 <sup>bbb</sup>
MCH	56.34±0.372	47.44±0.1674***	55.45±0.2518***	49.22±0.2793 <sup>aaa</sup>	51.1±0.2825 <sup>bbb</sup>
MCHC	42.08±0.315	36.44±0.3227***	41.73±0.4296***	38.09±0.05948 <sup>aaa</sup>	38.5±0.318 <sup>bbb</sup>

All values are expressed as mean±SEM and n=6. Data analysis done by one way analysis of variance followed by the Tukey multiple comparison tests and p<0.05 was considered as statistically significant. \*p<0.05, indicate normal control compared with disease control. \*p<0.05, indicate *Borassus flabellifer* and 5-Fluorouracil treatment group compared with disease control group. AOM: azoxymethane, DSS: Dextran sulfate sodium, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, WBC: White blood cell, RBC: Red blood cell, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, aaa: Low dose of *Borassus flabellifer*, bbb: High dose of *Borassus flabellifer*



Table 4: Effect of *Borassus flabellifer* on tissue antioxidants

Parameters	Control Group	Disease group (AOM/DSS)	Standard group (5-FU)	<i>Borassus flabellifer</i> (200 mg/kg)	<i>Borassus flabellifer</i> (400 mg/kg)
SOD	93.44±0.233	57.79±1.34***	93.13±0.3427###	72.21±1.16 <sup>aaa</sup>	80.9±1.553 <sup>bbb</sup>
GSH	9.742±0.039	6.298±0.057***	9.377±0.28###	7.22±0.139 <sup>aaa</sup>	8.27±0.061 <sup>bbb</sup>
Catalase	10.75±0.107	5.723±0.0345***	10.33±0.1713###	6.95±0.233 <sup>aaa</sup>	8.298±0.086 <sup>bbb</sup>
LOP	2.278±0.092	5.777±0.158***	2.335±0.084###	3.40±0.212 <sup>aaa</sup>	2.89±0.170 <sup>bbb</sup>

All values are expressed as mean±SEM and n=6. Data analysis done by one-way analysis of variance followed by the Tukey multiple comparison tests and p<0.05 was considered as statistically significant. \*p<0.05, indicate disease control compared with normal. #p<0.05, indicate *Borassus flabellifer* treatment extract group compared with disease control group. AOM: Azoxymethane, DSS: Dextran sulfate sodium, SOD: Superoxide dismutase, GSH: Glutathione, LOP: Lipid peroxidation, aaa: Low dose of *Borassus flabellifer*, bbb: High dose of *Borassus flabellifer*

CAT showed a substantial decrease in the disease group (5.723±0.0345) compared to the control (10.75±0.107). Treatment with *B. flabellifer*, especially at the 400 mg/kg dose (8.298±0.086), exhibited a significant increase, with similar levels observed in the standard group (10.33±0.1713). This suggests a potential enhancement of hydrogen peroxide neutralization by *B. flabellifer*. LOP, an indicator of oxidative damage, displayed a significant increase in the disease group (5.777±0.158) compared to the control (2.278±0.092). Treatment with *B. flabellifer*, particularly at the 400 mg/kg dose (2.89±0.170), showed a notable decrease, indicating a potential protective effect against oxidative damage.

## DISCUSSION

Colon cancer has witnessed a substantial surge in cases, becoming the most prevalent form of cancer globally [27]. The incidence rates indicate that around 4.6% of men (1 in 22) and 4.2% of women (1 in 24) will be diagnosed with CRC during their lifetime, with an annual worldwide occurrence of 1.23 million new cases [26].

The pathogenesis of colon cancer is closely associated with intestinal inflammation and ulcerative colitis [17]. The current research highlights *B. flabellifer*'s role in mitigating carcinogenesis in an AOM/DSS mice model. The study reveals significantly elevated serum levels of ALT, AST, ALP, WBC, platelet count, and total protein in AOM/DSS-induced mice compared to normal mice [29]. Additionally, lower serum levels of hemoglobin, RBC, MCV, MCH, and MCHC in the AOM/DSS group suggest AOM/DSS-induced hepatic damage. Notably, treatment with the hydroalcoholic extract of *B. flabellifer* demonstrates a significant reduction (p<0.05) in ALT, AST, ALP, WBC, platelet count, and total protein levels.

Consistent with prior research demonstrating the plant's anticancer activity, studies on *B. flabellifer* seed coat extracts indicate inhibitory effects on the HeLa cell line. Cytotoxicity assessment through the MTT assay underscores its potential in impeding HeLa cell growth. Further investigations reveal the plant's potent antioxidant activity against various types of free radicals, as evidenced by assays such as DPPH, FRAP, and ABTS *in vitro* (Jerry, 2018). Thus, the current study posits that the anticancer activity against colon cancer in mice can be attributed to the pharmacological properties derived from the plant's antioxidant and anti-inflammatory activities.

## CONCLUSION

Colon cancer is a prevalent Tumor of the digestive system that is difficult to identify early on and is the biggest cause of cancer mortality globally. One of the most difficult obstacles in the treatment of CRC is innate and acquired resistance to typical chemotherapeutic drugs. Although certain synthetic drugs are now used to treat colon cancer, they are insufficient and may have adverse effects. Recently, medicinal plants and their phytochemical contents have been proposed as therapeutic adjuvants in the prevention of colon cancer. In this regard, we evaluated the anti-colon cancer of *B. flabellifer*. Overall results observed that *B. flabellifer* shows more effective against colon cancer progression. However, this plant has positive effects toward AOM/DSS induce colon cancer pathogenesis. However, the effect of these plants

on colon cancer still needs to extend further investigation to study their molecular mechanism and clinical applications in colon cancer therapy. Nevertheless, more phytoconstituent identification and characterization, immunohistochemistry, and western blot analysis will be required to assess its suitability for use in the treatment of CRC.

## AUTHOR'S CONTRIBUTIONS

All the authors are involved in the review of literature, collection of data and preparation of the manuscript and also they were involved in reviewing and editing of the manuscript.

## CONFLICT OF INTERESTS

There is no conflict of interest for this research.

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