Evaluation of the antioxidant, anti-inflammatory, and antitumor properties of *Sabal* grown in Egypt

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Objective
Evaluation of in-vitro antioxidant, anti-inflammatory, and anticancer activities of alcoholic extracts of leaves and berries of *Sabal palmetto*, as well as in-vivo antitumor ability of alcoholic extract of berries of *S. palmetto* against Ehrlich ascites carcinoma is the aim of this study.

Materials and methods
Antioxidant properties of crude alcoholic extract of leaves and berries as well as two fractions of berries extract, ethyl acetate and butanol, were evaluated in-vitro compared with the standard materials, l-ascorbic acid (vitamin C) and butylated hydroxytoluene. The anti-inflammatory activity was investigated in-vitro using cyclooxygenase (COX)-1 and COX-2 inhibition assays. Moreover, in-vivo antitumor effect of *S. palmetto* alcoholic extract was evaluated using Ehrlich ascites carcinoma model. Data were presented as mean±SE, and data were analyzed by one-way analysis of variance test.

Results and conclusion
Crude extract from berries showed potent antioxidant activity compared with extract of leaves. Crude extract of berries was fractionated into two fractions: ethyl acetate and butanol. Ethyl acetate fraction showed good free radical scavenging activity, reducing capability, metal ion chelating activity, hydrogen peroxide scavenging activity, nitric oxide scavenging activity, and lipid peroxidation inhibition. Meanwhile, butanol fraction produced the highest superoxide anion scavenging activity and total antioxidant capacity. Anti-inflammatory activity of *S. palmetto* berries hydroalcoholic extract and its fractions showed weak COX-1 inhibition activity, whereas COX-2 was inhibited (100%), compared with celecoxib drug (72% at 1000 ppm). The ethyl acetate fraction of *S. palmetto* significantly reduced the viable Ehrlich cell count and increased nonviable count with amelioration of all hematological parameters. This amelioration reflected on increasing median survival time and significant increase ($P<0.05$) in lifespan. *S. palmetto* berries are candidate for intensive investigations as an alternative biological source for Saw palmetto.

Keywords:
anti-inflammatory, antitumor, Ehrlich ascites carcinoma, hematological parameters, Sabal palmetto

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Introduction

Many reactive species are found in the biological system, including reactive species centered on oxygen, nitrogen, and chlorine as well as sulfur molecules [1]. Although reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in many biological processes, some ROS work as cellular messengers in redox signaling. Moreover, the immune system uses ROS to attack and kill pathogens. When cell fails to equilibrate between generated ROS or RNS during biological process and the biological system's ability to detoxify or scavenge these reactive species or to repair the resulting damage, oxidative stress occurs. Chemically, oxidative stress is known as a remarkable increment in oxidizing species production or a significant reduction in the effectiveness of antioxidant defenses [2]. Free radicals or ROS are generated during respiration and cell-mediated immune functions. Reactive species like hydroxyl radical (OH), hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet-}$), hypochlorite radical, various lipid peroxides, nitric oxide ($NO^{\bullet}$), and peroxyl nitrite are able to react with macromolecules and micromolecules like lipid membrane, nucleic acids, proteins, and enzymes, leading to cellular damage or induce many diseases in humans, including cancer [3], Parkinson's disease [4], and Alzheimer's disease [5].

Sabal palmetto (Bartr.) is a small palm tree of the family Arecacea. *S. palmetto* also known as cabbage palm,

palmetto palm, cabbage palmetto, swamp cabbage, *Carolina palmetto*, and *Sabal palm. S. palmetto* is a low-growing, shrubby palm widely distributed in Egypt as an ornamental palm. Among all species of *Sabal*, *Sabal serrulata* (*Serenoa repens* or Saw palmetto) is used as a drug for treatment and prevention of prostate hyperplasia and nonbacterial prostatitis [6]. It has also been reported for its anti-inflammatory activity, antiandrogen properties, antiedema effects, and spasmolytic and smooth muscle relaxant activity [7]. It is also used in Folk medicine as an infusion to relieve irritated throat and symptoms of the common cold. The dried berries have been used as a menstrual drug product as well for the treatment of seborrhea, acne, and hair loss [8]. *S. serrulata* berries were also reported to treat lower urinary tract symptoms, most frequently owing to BPH. Moreover, it is used as an herbal medicine to treat a variety of conditions, including chronic pelvic pain, bladder and urinary disorders, and hormone imbalances [9]. *S. serrulata* have a good margin of safety [10]. Since the 1990s, Saw palmetto has been one of the top 10 selling herbal medicines in the world. Turnover of Saw palmetto preparations is likely to be 700 million dollars per annum [11]. This turnover is through many pharmaceutical preparations that contain Saw palmetto extract such as hair lotions for the treatment of hair loss, capsules for the treatment of hair loss, and ointments for the treatment of acne [8]. Several pharmaceutical preparations formed from *S. serrulata*-based over-the-counter drugs companies to treatment of BPH such as Permixon and Prostaserene [8].

Therefore, studying of another species of *Sabal*, *S. palmetto,* which has not been investigated before, will merit economical and pharmaceutical effects. In this study, we aimed to evaluate the antioxidant and anti-inflammatory activities of *S. palmetto* leaves and berries alcoholic extracts and berries alcoholic extract fractions. In addition to the antitumor effect of ethyl acetate fractions of berries, alcoholic extract was investigated to prove its effects to be incorporated in advanced in-vivo studies.

Materials and methods

Materials

Plant materials and extraction

Leaves and berries of *S. palmetto* were collected from Zohreya Botanical Garden, Cairo, Egypt, and authenticated by Dr M. Elgebally, former researcher of botany at National Research Center. A voucher specimen was deposited at the herbarium of Zoherya Botanical Garden. One kilogram of *S. palmetto* berries and leaves was extracted thrice with ethanol (70%) to yield 150 and 50 g, respectively. The alcohol extract of berries organ was then fractionated with ethyl acetate and butanol.

Chemicals

Potassium ferricyanide [$K_3Fe(CN)_6$]; ferric chloride ($FeCl_3$); trichloroacetic acid; 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine); ferrous chloride ($FeCl_2$); nicotinamide adenine dinucleotide (NADH); phenazine methosulphate (PMS); nitroblue tetrazolium (NBT); sodium nitroprusside (SNP); sulfanilamide; ortho-H_3PO_4; naphthylethylene diamine dihydrochloride linoleic acid; polyoxyethylenesorbitan monolaurate (Tween-20); peroxidase; hydrogen peroxide; 2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid, diammonium salt (ABTS); 1,1-diphenyl-2-picryl-hydrazyl (DPPH); l-ascorbic acid (vitamin C); butylated hydroxytoluene (BHT); leuco-2,7-dichlorofluorescien diacetate; hematin; arachidonic acid; and cyclooxygenases enzymes (COX-1 from sheep, EC. 1.14.99.1 or COX-2) were purchased from Sigma-Aldrich (Schnelldorf, Germany). Ammonium thiocyanate was purchased from E. Merck (Frankfurter, Darmstadt, Germany). All chemical and solvents used are of analytical grade.

Methods

Determination of chemical composition of *S. palmetto*

Total polyphenols content

The total phenolic content of *S. palmetto* extracts and fractions was determined by Folin–Ciocalteu method as described by Singleton *et al.* [12]. The concentration of phenolics was expressed as mg gallic/g extract.

Total flavonoid contents

Flavonoid contents of *S. palmetto* extracts and fractions were determined according to the method of Zhishen *et al.* [13]. The results were expressed as mg quercetin equivalents/g extract.

Total carbohydrates

Complete acid hydrolysis of *S. palmetto* berries crude extract was carried out according to the modified method by Fisher and Dörfel [14]. Total carbohydrate was determined in the extract using phenol-sulfuric acid method by DuBois *et al.* [15].

Qualitative examination of the hydrolysis products

The hydroalcoholic extract of the berries was dissolved in water and precipitated by acetone. The precipitate was co-chromatographed on Whatman no. 1 filter paper, using the solvent system *n*-butanol–acetone–water

(4 : 5 : 1) against authentic samples of d-galactose, d-mannose, d-glucose, d-glucouronic acid, and l-rhamnose and some pentoses and disaccharides as reference substances [16].

Quantitative determination of the hydrolysis products

Quantitative determination of the hydrolysis sugars was done according to the modified method of Wilson [16]. The quantities of sugars were determined by comparison with appropriate standard curves constructed under the same conditions.

Antioxidant properties evaluation

Reduction capability

The reduction capability of *S. palmetto* extracts or fractions was determined according to the method of Oyaizu [17]. The different concentrations in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000*g* (MSE Mistral 2000; UK, and serial no.: S693/02/444). The upper layer of solution (2.5 ml) was mixed with methanol (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Metal chelating effect

The chelating effect of *S. palmetto* extracts, fraction, and standards against ferrous ions was estimated by the method of Dinis *et al.* [18]. In summary, concentrations of extracts and standards were added to a solution of 2 mmol/l $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml), and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\text{Inhibition}(\%) = \frac{A_0 - A_1}{A_0} \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of samples and standards [19].

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging (SOR) activity of *S. palmetto* extracts and fractions was based on the method described by Liu *et al.* [20] with slight modifications [19]. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT. In this experiments, the superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mmol/l, pH 8.0) containing 1 ml of NBT (50 μmol/l) solution, 1 ml NADH (78 μmol/l) solution, and 1 ml of sample solutions, which were mixed in different concentrations. The reaction was started by adding 1 ml of PMS solution (10 μmol/l) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. The inhibition percentage of SOR generation was calculated using the following formula:

$$\text{Inhibition}\% = \frac{A_0 - A_1}{A_0} \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of extracts and standards.

Scavenging of hydrogen peroxide ability

The ability of *S. palmetto* samples and standards to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch *et al.* [21]. A solution H_2O_2 (40 mmol/l) was prepared in phosphate buffer (pH 7.4). Samples and standards concentrations in methanol were added to H_2O_2 solution (0.6 ml, 40 mmol/l). Absorbance of H_2O_2 at 230 nm was determined after ten min against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of samples and standard compounds was calculated using the following equation:

$$H_2O_2(\%) = \frac{A_0 - A_1}{A_0} \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of samples and standards [19].

Nitric oxide radical scavenging activity

$NO^{\bullet}$ radical scavenging activity of *S. palmetto* extracts and fractions was determined by using a system of generating NO from SNP. $NO^{\bullet}$ generated from SNP in aqueous solution at physiological pH reacts with oxygen to produce nitrite ions, which were measured by the Greiss reagent as stated by Marcocci *et al.* [22], which constitutes 1% sulfanilamide in 5% ortho-H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride. The reaction mixture (2 ml) containing various concentrations of different tested compounds and SNP (final concentration, 10 mmol/l) in PBS, pH=7.4, were incubated at 25°C for 150 min After incubation, 1 ml each reaction mixtures was removed and diluted with 1-ml Greiss reagent. The

absorbance of these solutions was measured at 540 nm against the corresponding blank solution.

Lipid peroxidation inhibition activity

Inhibition of lipid peroxidation in ammonium thiocyanate system of *S. palmetto* fractions and standard was determined according to the method of Gülçin *et al.* [23]. A pre-emulsion was prepared by mixing 175 μg Tween 20, 155 μl linoleic acid, and 0.04 mol/l potassium phosphate buffer (pH 7.0). Overall, 1 ml of sample in 99.5% ethanol was mixed with 4.1 ml linoleic emulsion, 0.02 mol/l phosphate buffer (pH=7.8), and distilled water (pH=7.9). The mixed solutions of all tested samples (21 ml) were incubated in screw cap tubes under dark conditions at 40°C at certain time intervals. To 0.1 ml of this mixture was pipetted and added with 9.7 ml of 75% and 0.1 ml of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 ml of 0.02 mol/l ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. All test data were the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Inhibition}(\%) = \frac{A_0 - A_1}{A_0} \times 100,$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of samples and standard compounds.

$ABTS^+$ radical scavenging activity

$ABTS^+$ radical scavenging activity of *S. palmetto* extracts and fractions and standards were measured according to the method described by Miller and Rice-Evans [24] and Arnao *et al.* [25]. Each tested sample was prepared in five concentrations (50, 100, 250, 500, and 1000 μg/ml). Both of vitamin C and BHT were used as standard materials and prepared with the same concentrations. Exactly 0.2 ml of peroxidase (4.4 U/ml), 0.2 ml of H_2O_2 (50 μmol/l), 0.2 ml of ABTS (100 μmol/l), and 1 ml methanol were mixed and kept in the dark for 1 h to form a bluish green complex after the addition of 1 ml of tested samples and standard at different concentrations. The absorbance at 734 nm was measured to represent the total antioxidant capacity and then was calculated as follows:

$$ABTS^+ \text{ radical scavenging activity } (\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100,$$

where A_{samples} was the absorbance of the samples and standards, and A_{control} was the absorbance of control.

$DPPH^\bullet$ radical scavenging activity

The free radical scavenging activity of *S. palmetto* samples was measured by $DPPH^\bullet$ using the method of Yamaguchi *et al.* [26]. Overall, 1 ml of $DPPH^\bullet$ solution (0.1 mmol/l $DPPH^\bullet$ in methanol) was added to 3 ml of each concentration of samples and standard materials. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The control sample was prepared with the same procedure without sample. The $DPPH^\bullet$ radical concentration in the reaction medium was calculated from the following equation:

$$DPPH^\bullet \text{ radical scavenging effect}(\%) = 100 - \left(\frac{A_0 - A_1}{A_0} \times 100\right),$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the samples.

In-vitro anti-inflammatory activity

The cyclooxygenase inhibition assay was performed according to a modified method of Larsen *et al.* [27]. The oxidation of leuco-dichlorofluorescein in the presence of phenol by the hydroperoxide formed in the cyclooxygenase reaction can be used as a sensitive spectrophotometric assay for prostaglandins (PG) synthase activity, and the reaction was recorded spectrophotometrically at 502 nm. The anti-inflammatory activity of *S. palmetto* extracts and fractions was evaluated by comparison with celecoxib drug.

In-vitro anticancer activity using cell line assay

In-vitro anticancer ability of sabal berries crude extract was investigated against human prostate cancer (PC3) and human white breast adeno-carcinoma (MCF7). Cell viability of MCF7 and PC3 were evaluated by the mitochondrial-dependent reduction of yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide to purple formazan [28]. Cells were suspended in RPMI 1640 medium. Sabal extracts were tested with different concentrations to give a final concentration of 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 μg/ml [29,30]. The absorbance was measured using a microplate multiwell reader (model 3350; Bio-Rad Laboratories Inc., Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. The percentage of change

in viability was calculated according to the following formula:

Reading of extract/reading of negative control -1×100.

Antitumor activity of *S. palmetto* berries alcoholic extract against Ehrlich ascites carcinoma in mice

Ethical issues

This study was a part of the project entitled 'New drug discovery for breast and prostate cancers from Egyptian medicinal plants and polysaccharides derived from natural sources'. This study was approved by Medical Research Ethics Committee, National Research Centre, Egypt (under registration no. 6/014).

Acute toxicity study

The acute toxicity test for *S. palmetto* berries alcoholic extract was carried out to evaluate any possible toxicity. Albino female mice (n=8) were tested by oral administration of different doses of the extract by increasing or decreasing the dose, according to the response of the animal [31]. The dosing patron was started from 500 mg/kg and increased to reach 6000 mg/kg by increasing the dose at a rate of 500 mg/kg body weight whereas the control group received only the normal saline. Death of half of examined animals (LD_{50}) was recorded at 4800 mg/kg body weight which was calculated using BioStat program (BioStat 2009 Build 5.8.4.3). Therefore, the selected dose to study the in-vivo antitumor activity of Sabal berries crude extract was 480 mg/kg body weight/day as the 10th of the LD_{50} (according to the study of Garg *et al.* [32], and Ghosh [33]).

Tumor cell

Ehrlich ascites carcinoma (EAC) cells were obtained from National Cancer Institute, Cairo, Egypt, and they were used at a concentration of 2×10^6 cell/mouse.

Experimental layout

Albino female mice (70 mouse) were obtained from animal house of National Research Centre and ranged in weight from 20 to 25 g. They were fed on standard diet and water *ad libitum*. Animals were maintained under normal laboratory conditions 1 week before experimental period and kept in standard polypropylene cages at room temperature of 25–30°C and 60–65% relative humidity for adaptation. Mice were classified into five groups, each of them contained 14 albino female miceGroup I: mice received the vehicle (saline solution) orally for 10 consecutive days, and they served as a negative control group. Group II: mice were received saline orally for 10 consecutive days, and they were injected with EAC (2×10^6 cell/mouse) and served as tumor-bearing group. Group III: mice were injected with EAC (2×10^6 cell/mouse) and were incubated for 24 h, and then they were force fed with a reference drug, 5-fluorouracil with recommended dose 20 mg/kg body weight/day, for 10 consecutive days [34]. Group IV: mice were treated orally with *S. palmetto* berries alcoholic extract at a dose of 480 mg/kg body weight (acted 0.10 of determined LD_{50}) for 10 consecutive days and served as a positive control group. Group V: mice were injected with EAC (2×10^6 cell/mouse) and were incubated for 24 h, then they were administered *S. palmetto* berries alcoholic extract at dose of 480 mg/kg body weight for 10 consecutive days and served as a treated group. After fasting for 18 h, blood samples were collected from cardiac puncture from six mice/group only, after 10 days of extract and 5-fluorouracil drug administration, for estimation of hematological parameters. Eight mice were kept alive to check the increase in lifespan of the tumor-bearing hosts [35].

Determination of tumor volume and weight

Mice were dissected, and the ascetic fluid was collected from peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weighed immediately [36].

Estimation of viable and nonviable tumor cell count

The ascetic fluid was taken in white blood cell pipette and diluted 100 times. A drop of diluted suspension was paled on the Neubauer counting chamber and the cell were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye are viable and those that took up the stain were nonviable. These viable and nonviable cell counts were estimated by the following equation [36]:

$$\text{Cell count} = \frac{\text{Number of cell} \times \text{dilution}}{\text{Area} \times \text{thickness film}}.$$

Determination of median survival time and percentage increase in lifespan

Increase in lifespan was recorded for monitoring of mortality, and median survival time was also estimated by the following formula: increases of lifespan%= $(T\text{-}C/C)\times100$, where T is the number of days where treated animals survived, whereas C is number of days control mice survived [36].

Determination of solid tumor

The solid tumor of mice was estimated according to Kuttan *et al.* [37]. The tumor mass was measured from the 11th day of tumor induction. The measurement

was carried out every five day for 30 days. The volume of tumor mass was calculated using the following formula:

$$V = 4/3 \times \pi r^2,$$

where r is the mean of r_1 and r_2, which are independent radii of the tumor mass.

Estimation of hematological parameters

Collected blood samples were used for determination of hematological parameters according to Dacie and Lewis [38], including hemoglobin, total leukocyte count, red blood cell count, packed cell volume, platelet count, and differential white blood cell.

Estimation of liver enzymes activities

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were spectro photometrically in serum measured according to Reitman and Frankel [39].

Statistical analysis

All data were mentioned as median±SE. Data were analyzed by analysis of variance one-way (in-vitro study n=3 replicates, and in-vivo study n=6 replicates) and P value of less than 0.05.

Results

Chemical composition of *S. palmetto*

Total phenol and flavonoids content of S. palmetto

The total phenol and flavonoids content of crude alcoholic extract of berries was 14.30±0.03 mg gallic acid/g extract and 5.17±0.25 mg quercetin/g extract, which is higher than that of crude leaves extract (6.30±0.02 mg gallic/g extract and 2.35±0.15 mg quercetin/g extract). Both fractions of berries extract, ethyl acetate and butanol, contained 16.40±0.17 and 12.70±0.16 mg gallic acid/g extract and 5.81±0.12 and 4.13±0.08 mg quercetin/g extract, respectively.

Total carbohydrates content of berries crude extract

Total carbohydrate content was determined to be 94% in *S. palmetto* berries.

Qualitative determination of carbohydrates contents of berries crude extract

S. palmetto crude extract of berries was investigated against sugar authentic samples, and the extract was showed to contain mannose, galactose, glucose, rhamnose, and uronic acids derivatives like glucouronic acid and galactouronic acid in addition to uronic acid polymers.

Quantitative determination of carbohydrates contents of berries crude extract

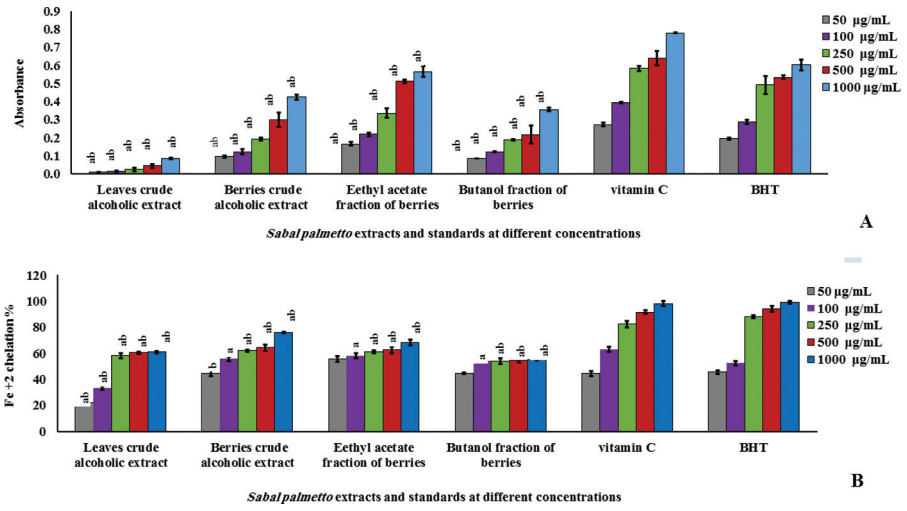
Monosaccharide constituents of *S. palmetto* berries (% w/w) were tentatively quantified to be 17% d-glucose, 37% d-galactose, 5% rhamnose, 12% d-mannose, 10% d-glucuronic acid, 14% d-galactouronic acid, and 5% uronic acids containing polymer.

Antioxidant properties

Reducing power

Data in Fig. 1a showed that crude berries extract showed moderate reducing power, and this activity

Figure 1



Total ferric reducing power (FRAP) (part A) and Fe^{2+} chelation activity (part B) of *Sabal palmetto* leaves and berries crude alcoholic extracts as well as ethyl acetate and butanol fractions of berries at different concentrations (50–1000 µg/ml) compared with standard materials, vitamin C and BHT (BHT: butylated hydroxytoluene). Data are presented as mean±SE. One-way analysis of variance was used for data analysis (n=3, P<0.05). Data are followed with small letter; a, means significant difference with vitamin C; b, means significant difference with BHT.

was potent than leaves crude extract ($P<0.05$). When crude berries were fractionated into ethyl acetate and butanol fractions, ethyl acetate fraction was more effective than butanol fraction. The highest reducing power was represented by ethyl acetate fraction of berries which was near to BHT, whereas the lowest value was recorded by leaves crude extract, compared with reducing power of vitamin C or BHT.

Ferrous ions (Fe^{2+}) chelating effect

Data presented in Fig. 1b showed that Fe^{2+} ion chelation effect was concentration dependent for all tested materials ($P<0.05$). Berries extract showed high Fe^{2+} ion chelation ability, compared with leaves extract with respect to vitamin C and BHT. Ethyl acetate fraction of berries captured Fe^{2+} ion higher than butanol fraction. The IC_{50} of *S. palmetto* extracts Fe^{2+} ion chelation ranged between 317.29±7.01 μg/ml for leaves alcoholic and 10.43±0.57 μg/ml for ethyl acetate fraction of berries alcoholic extract (data in Table 1). IC_{50} of ethyl acetate fraction was much lower than that of vitamin C and BHT (65.18±1.97 and 68.85 ±3.10 μg/ml, respectively).

Superoxide radical scavenging effect

Alcoholic extract of leaves or berries inhibited generation of O_2^- radical in PMS-NADH-NBT system moderately compared with two standard compounds (Fig. 2a) ($P<0.05$). Two fractions of berries extract showed superiority to butanol fraction on ethyl acetate. Butanol fraction showed O_2^- scavenging percentage ranged from 44.25±1.20 to 83.29±1.20% at 50–1000 μg/ml, compared with vitamin C (45.12±2.20 to 97.75±1.32%) and BHT (48.25±1.37 to 98.11±2.00) at the same concentrations. The IC_{50} of *S. palmetto* extracts for O_2^- radical scavenging ranged between 1123.00± 23.00 μg/ml for leaves alcoholic extract and 62.50± 1.00 μg/ml for butanol fractions (data in Table 1). IC_{50} of butanol is close to these of vitamin C and BHT (52.36±1.10 and 48.14±1.86 μg/ml, respectively).

Hydrogen peroxide scavenging effect

Berries crude alcoholic extract represented the highest H_2O_2 scavenging, higher than that represented by leaves alcoholic extract. The ethyl acetate fraction of berries extract reached the nearest values to standards at the 500 and 1000 μg/ml [82.64±0.60 and 90.15±1.85 compared with two standard compounds; vitamin C (86.77±1.31 and 95.53±0.20%), and BHT (84.00±1.50 and 93.53±1.50%)] at the same concentrations (Fig. 2b). The IC_{50} of *S. palmetto* extracts ranged between 491.66±11.00 μg/ml for leaves alcoholic and 192.82±4.80 μg/ml for berries alcoholic extract, compared with these of vitamin C and BHT (15.17±0.83 and 14.83±1.40 μg/ml, respectively) (data in Table 1).

Inhibition of nitrite formation

S. palmetto inhibited the $NO^{\bullet}$ liberation from SNP through its effect as nitric oxide radical scavenger. $NO^{\bullet}$ radical scavenging activity was much higher in the crude alcoholic extract of berries, with 72.51±1.50% at 1000 μg/ml in comparison with 60.43±1.20% in leaves extract at1000 μg/ml. Fractionation crude berries extract into ethyl acetate and butanol did not increase $NO^{\bullet}$ radical scavenging significantly ($P<0.05$) (Fig. 3a). The IC_{50} of $NO^{\bullet}$ radical scavenging of *S. palmetto* extracts ranged between 338.13±8.05 μg/ml for leaves alcoholic extract and 47.15±2.03 μg/ml for ethyl acetate fraction, compared with IC_{50} of vitamin C and BHT (53.39±2.29 and 53.20± 2.80 μg/ml, respectively) (data in Table 1).

Inhibition of lipid peroxidation

The berries extract showed superior lipid peroxidation effect than the leaves extract ($P<0.05$) (Fig. 3b). Among two fractions of the berries extract, the ethyl acetate fraction was the most effective, as it inhibited

Table 1 IC_{50} (μg/ml) of *Sabal palmetto* leaves and berries extracts and ethyl acetate and butanol fractions of berries alcoholic extract compared with reference materials, vitamin C and butylated hydroxytoluene

Groups	DPPH scavenging	BTS^+ scavenging	O^{2-} scavenging	H_2O_2 scavenging	NO scavenging	Lipid peroxidation	Metal chelation
Leaves	982.39±18.40[a,b]	741.00±15.50[a,b]	1123.00±23.00[a,b]	491.66±11.00[a,b]	338.10±8.05[a,b]	245.85±8.80[a,b]	317.29±7.01[a,b]
Berries	412.45±12.40[a,b]	234.12±6.00[a,b]	702.20±10.00[a,b]	192.82±4.80[a,b]	59.41±3.40	275.67±9.40[a,b]	82.04±1.83[a,b]
Ethyl acetate	293.05±12.00[a,b]	437.62±9.95[a,b]	271.84±5.00[b]	244.17±11.83[a,b]	47.10±2.03[a,b]	63.93±3.30[a,b]	10.43±0.57[a,b]
Butanol	1275.80±50.15[a,b]	339.09±8.35[a,b]	62.50±1.00[a,b]	262.04±5.88[a,b]	98.66±3.59[a,b]	178.77±8.00[a,b]	171.40±6.00[a,b]
Vitamin C	41.41±1.57	79.23±2.21	52.36±1.10	15.17±0.83	53.38±2.29	74.03±2.83	65.17±1.97
BHT	44.53±2.53	80.45±2.42	48.14±1.86	14.83±1.40	53.20±2.80	74.58±3.70	68.85±3.10

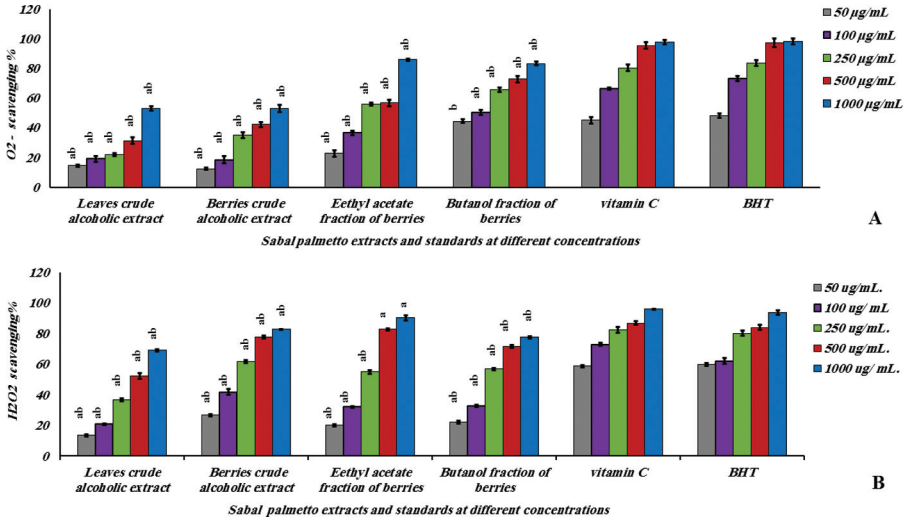
Data presented as mean±SE; BHT, butylated hydroxytoluene; BTS^+, 2,20-azinobis (3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 1,1 diphenyl-2-picryl-hydrazyl free radical; H_2O_2, hydrogen peroxide; One-way analysis of variance was used for data analysis ($n=3$, $P<0.05$); Data are followed with small letter; [a]Mean significant difference with vitamin C; [b]Mean significant difference with BHT.

lipid peroxidation by 47.75±1.46 to 81.35±1.70% at 100–1000 µg/ml, whereas butanol fraction produced less inhibitory effect (38.78±0.70 to 66.52±1.18% at 50–1000 µg/ml), compared with vitamin C and BHT. The IC_{50} of *S. palmetto* extracts in lipid peroxidation inhibition ranged between 275.67±9.40 µg/ml for leaves alcoholic to 63.93±3.30 µg/ ml for ethyl acetate fraction of berries extract, compared with IC_{50} of vitamin C and BHT (74.00±2.83 and 74.58±3.70 µg/ml, respectively) (data in Table 1).

DPPH• radical scavenging effect

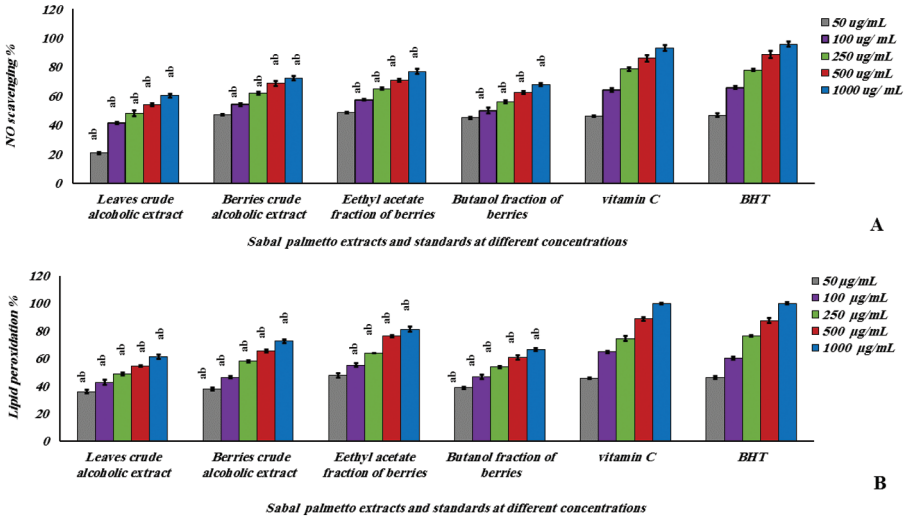
Berries alcoholic extracts scavenged DPPH radicals in concentration-dependent manner whereas leaves extract was the weak radical scavenger (Fig. 4a). The ethyl acetate fraction had a potent scavenging effect, which was magnified with increasing the concentration from 50 µg/ml (20.04±2.24%) to 1000 µg/ml (88.41±0.99%), whereas butanol fraction scavenged radicals by 44.92±1.32% at the maximum concentration. The smallest IC_{50} of *S. palmetto* extracts as free radicals scavenging was 293.45±12.00 µg/ml for ethyl acetate

Figure 2



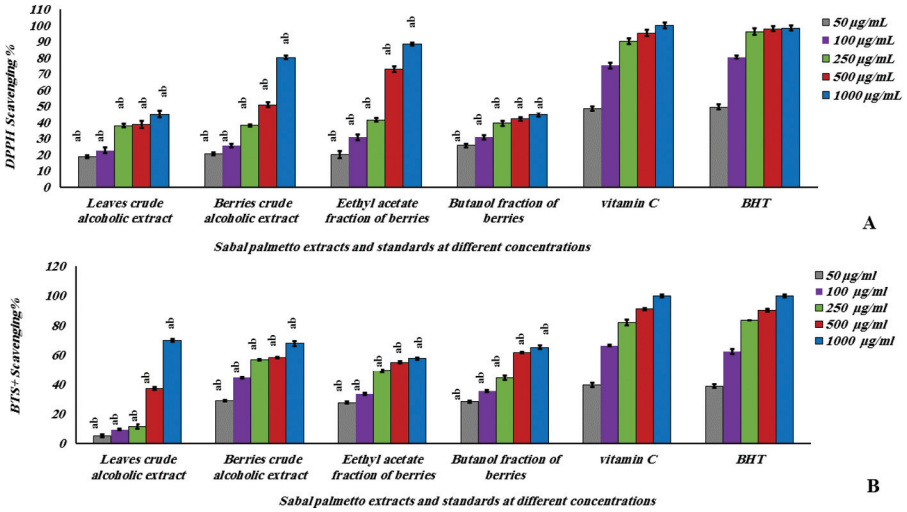
O_2^- scavenging (part A) and H_2O_2 scavenging (part B) activities of *Sabal palmetto* leaves and berries crude alcoholic extracts as well as ethyl acetate and butanol fractions of berries at different concentrations (50–1000 µg/ml) compared with standard materials, vitamin C and BHT. Data are presented as mean±SE. One-way analysis of variance was used for data analysis (n=3, P<0.05). Data are followed with small letter; a, means significant difference with vitamin C; b, means significant difference with BHT. BHT, butylated hydroxytoluene.

Figure 3



NO scavenging (part A) and lipid peroxidation (part B) activities of *Sabal palmetto* leaves and berries crude alcoholic extracts as well as ethyl acetate and butanol fractions of berries at different concentrations (50–1000 µg/ml) compared with standard materials, vitamin C and BHT (BHT: butylated hydroxytoluene). Data are presented as mean±SE. One-way analysis of variance was used for data analysis (n=3, P<0.05). Data are followed with small letter; a, means significant difference with vitamin C; b, means significant difference with BHT.

Figure 4



Free radicals scavenging activities of *Sabal palmetto* leaves and berries crude alcoholic extract at different concentrations (100–1000 µg/ml) compared with standard materials, vitamin C and BHT against DPPH (part A), ABTS (part B). Data are presented as mean±SE. One-way Analysis of variance was used for data analysis (n=3, P<0.05). Data are followed with small letter; a, means significant difference with vitamin C; b, means significant difference with BHT. BHT, butylated hydroxytoluene; DPPH, 1,1 diphenyl-2-picryl-hydrazyl free radical; BTS^+, 2,20-azinobis (3-ethylbenzthiazoline-6-sulfonic acid).

fraction, whereas the biggest one 1275.69±50.15 µg/ml for butanol fraction, compared with vitamin C (41.41± 1.57 µg/ml) and BHT (44.54±2.53 µg/ml) (data in Table 1).

$ABTS^+$ radical cation scavenging effect

The $ABTS^+$ scavenging ability was much higher in the berries extract than that of leaves extract with respect to vitamin C and BHT (Fig. 4b). Berries extract had the $ABTS^+$ scavenging much higher than their fractions, ethyl acetate or butanol. The IC_{50} for the $ABTS^+$ scavenging ability of *S. palmetto* extracts ranged between 724.03±15.50 µg/ml for leaves alcoholic and 234.00±6.00 µg/ml for berries alcoholic extract, compared with IC_{50} of vitamin C and HBT (80.44± 2.41 and 79.22±2.21 µg/ml, respectively) (data in Table 1).

Anti-inflammatory activity

S. palmetto significantly inhibited COX-2 in a dose-dependent manner (Fig. 5a). Celecoxib, the reference drug, showed COX-1 inhibition percentage from 52.50±2.50% at 100 µg/ml to 67.82±2.20% with 1000 µg/ml. Celecoxib was effective on COX-2 and recorded inhibition percentage from 41.23 ±1.77% at 100 µg/ml to 72.49±2.20% at 1000 µg/ml. All *S. palmetto* materials inhibited COX-2 activity to reach 100% inhibition at the highest concentration, 1000 µg/ml. COX-2 inhibition percentage was much higher in berries extract, with 49.53±1.51% at 100 µg/ml to 100.00±0.25% at 1000 µg/ml, than that in leaves extract (18.31±1.05 to 100±0.34% at the same concentration). Ethyl acetate fraction of berries showed potent inhibitory effect against COX-2, and it reached 100% inhibition percentage at two concentrations (500 and 1000 µg/ml) with low IC_{50} of 53.21 µg/ml, whereas leaves and berries crude extracts as well as butanol fraction showed the same inhibition percentage at 1000 µg/ml but with IC_{50} of 366.14, 143.75, and 244.94 µg/ml, respectively, with respect to the IC_{50} of celecoxib 211.31 µg/ml.

On the contrary, all *S. palmetto* extracts showed selectivity against COX-1, represented as a weak inhibition effect on COX-1, as compared with reference drug, celecoxib. They reached the highest inhibitory effect (around 50%) at 1000 µg/ml which was the lowest inhibitory level of celecoxib (at 100 µg/ml) (Fig. 5b). The lowest inhibitory effect on COX-1 was observed with butanol fraction (IC_{50}, 1867.57 µg/ml) followed by ethyl acetate fraction and berries alcoholic extract (909.52 and 798.74 µg/ml, respectively) and then leaves alcoholic extract (IC_{50}, 751.57 µg/ml). Inhibition percentage of different tested materials ranged from 8.80 to 56% for leaves extract, 9.50 to 52.90% for berries alcoholic extract, 5.80 to 64% for ethyl acetate fraction, and 0.94 to 23.50% for butanol fraction. The ethyl acetate fraction was the effective one to inhibit COX-2 (IC_{50}, 53.21 µg/ml) with lowest inhibitory effect on COX-1 (IC_{50}, 909.52 µg/ml).

In-vitro anticancer activity of *S. palmetto* berries crude extract using cell line assay

Cytotoxic effect of Sabal berries crude extract was determined using human prostate cancer (PC3) and human white breast adenocarcinoma (MCF7) using cell viability assay. The extract at 100 ppm showed killing percentage against MCF7 cells reached to 34.50% with IC_{50} of 137.60 μg/ml, and 6.20% against PC3 cells.

Antitumor activity of crude alcoholic extract of *S. palmetto* berries against Ehrlich ascites carcinoma in mice

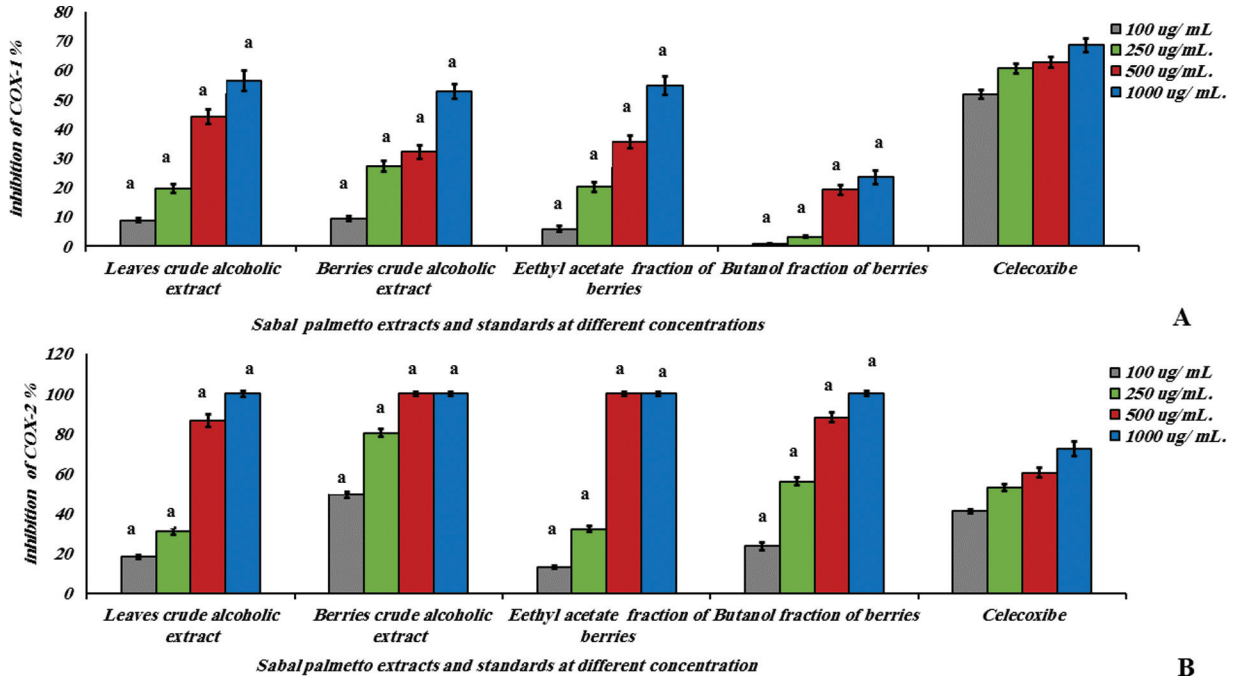
Viable and nonviable tumor cell count

Ten days after the treatment, the number of carcinoma cells was increased to reach 10.0×10^7 cell/ml, whereas treating animals with 5-fluorouracil significantly reduced the total cell count to 4.9×10^7 cell/ml. Cell count was much lower in the animals treated with Sabal fraction with, 3.0×10^7 cell/ml, in comparison with 10.0×10^7 cell/ml in tumor-bearing group (Table 2). The improved effect of *S. palmetto* extract on tumor-bearing mice was more pronounced than that of 5-fluorouracil drug. In parallel, the reduction of carcinoma cell growth was accompanied with reduction of viable cell count to 0.79×10^7 cell/ml for tumor-bearing mice force fed with *S. palmetto* extract. This observed effect was reflected in the great suppression on total number of nonviable cell count that increased to be 2.2×10^7 cell/ml by force feeding with *S. palmetto* extract (Table 2).

Hematological parameters

The ameliorative effect of Sabal extract on tumor cell count was also observed for recorded hematological parameters. They significantly ($P<0.05$) reduced the

Figure 5



Cyclooxygenases inhibition activity of *Sabal palmetto* leaves and berries crude alcoholic extracts as well as ethyl acetate and butanol fractions of berries at different concentrations (100–1000 μg/ml) compared with standard materials, celecoxib, COX-1 (part A) and COX-2 (part B). Data are presented as mean±SE. One-way Analysis of variance was used for data analysis ($n=3$, $P<0.05$). Data are followed with small letter; a, means significant difference with celecoxib.

Table 2 Effect *S. palmetto* berries alcoholic extract on viable and nonviable tumor cell count

Parameters	Tumor-bearing group	FU-treated group	*S. palmetto* treated group
Total cell count ($\times10^7$ cell/ml)	10.00±1.10	4.90±0.98*	3.00±0.50*
Viable cell count ($\times10^7$ cell/ml)	9.20±1.10	1.40±0.09*	0.80±0.40**
Nonviable cell count ($\times10^7$ cell/ml)	0.80±0.50	3.50±0.60**	2.20±0.60**
Viable cell (%)	97.20±1.60	18.00±0.70**,a	20.50±0.60**,a
Nonviable cell (%)	2.80±0.90	82.00±0.70**,b	79.60±0.80**,b

Data are presented as mean±SE; Analysis of variance one-way was used for data analysis followed by post-hoc test for multiple comparisons ($n=6$, $P<0.05$); *Mean significant level as compared with tumor-bearing mice group. Groups have the same letter have not significant changes in between them.

deleterious effect of carcinoma cells on hemoglobin concentration to change their values to 10.60±1.06 mg/dl for *S. palmetto* extract treated group as compared with tumor-bearing mice group (6.53±0.96 mg/dl) as mentioned in Table 3. The ameliorative effect of Sabal extract was higher than that of 5-fluorouracil on the hemoglobin. This preferable effect was also recorded for the red blood cell count which was $8.31\pm0.95\times10^6$ cell/mm^3 in mice force fed with *S. palmetto* extract as compared with tumor-bearing mice group ($7.24\pm1.11\times10^6$ cell/mm^3). On the contrary, packed cell volume and platelet count were reduced to 31.32±1.52 mm and 100.00×1000±1.60 cell/mm^3, respectively, in case of tumor-bearing mice, whereas they were improved with treating animals for 10 consecutive days with *S. palmetto* extract to reach 36.23±1.35 mm for packed cell volume as well as 232.50±1.47×1000 cell/mm^3 for platelets count. This repairing effect was produced also for the deferential white blood cell especially for neutrophils and lymphocytes percentages. Neutrophils and lymphocytes percentage reached 45.00±0.68 and 2.00±0.26%, respectively, for tumor-bearing mice treated with *S. palmetto* extract (Table 3). In comparison with 5-fluorouracil treated group, *S. palmetto* treated group showed hematological parameters healthier than these of 5-fluorouracil one. The same assessments were conducted for the positive controls administered sabal extract only to determine any negative effect on animals as a part of safety parameters. All recorded parameters showed that there is no deleterious effect on animals and they were enhanced by extract administration as mentioned in Table 3.

Table 3 Potential effect of *S. palmetto* alcoholic extract on hematological parameters

Parameter	Hb concentration	RBC's count	PCV	Platelets count	WBC count	Deferential white blood cells				
						Neutrophil	Basophiles	Lymphocyte	Monocytes	Eosinophil
Negative control	11.50 ± 1.06^{a}	11.12 ± 1.00^{b}	41.12±1.25	300.00 ± 1.41^{h}	7.00 ± 0.98^{m}	25.63 ± 0.69^{k}	65.00 ± 1.02^{p}	3.41 ± 1.24^{y}	4.00±0.78	1.96 ± 0.30^{t}
Tumor-bearing mice	$6.53\pm0.96^{**}$	$7.24\pm1.11^{**,s}$	$31.32\pm1.52^{*}$	$100.00\pm1.61^{**}$	$11.41\pm1.00^{*}$	$60.00\pm0.84^{**}$	$29.00\pm0.67^{**}$	$5.50\pm0.13^{**}$	$3.00\pm0.09^{*,r}$	$2.50\pm0.14^{*}$
FU-treated mice	$7.11\pm1.06^{**}$	$7.53\pm0.97^{**,s}$	$38.54\pm2.16^{*,u}$	$185.00\pm1.86^{**}$	$9.24\pm1.08^{**}$	$36.33\pm2.11^{*}$	56.24 ± 1.46	$4.16\pm0.74^{*}$	$3.27\pm0.85^{*,r}$	$0.00\pm0.04^{**}$
Control of *S. palmetto*	$13.00\pm1.11^{*}$	10.65 ± 1.16^{b}	$46.00\pm0.97^{*}$	296.77 ± 0.79^{h}	$6.80\pm0.28^{**,m}$	$28.00\pm1.17^{*,k}$	65.00 ± 1.75^{p}	3.00 ± 0.21^{y}	$2.00\pm0.10^{**}$	2.00 ± 0.14^{t}
S. palmetto treated mice	10.60 ± 1.06^{a}	$8.31\pm0.95^{*}$	$36.23\pm1.35^{*,u}$	$232.50\pm1.47^{*}$	7.00 ± 0.97^{m}	$45.00\pm0.68^{**}$	$52.00\pm1.84^{*}$	$2.00\pm0.26^{**}$	$3.00\pm0.05^{*,r}$	$0.00\pm0.03^{**}$

Data are presented as mean±SE; One-way analysis of variance was used for data analysis followed by post-hoc test for multiple comparisons (n=6; $P<0.05$). Tumor-bearing mice were compared with the negative control group whereas treated groups were compared with tumor-bearing group; FU, 5-flourouracil; Hb, hemoglobin (mg/dl); PCV, packed cell volume (mm); RBC's, red blood cell count (×million/mm^3); WBC, white blood cell (×1000/mm), platelets count (×1000/mm); *Mean significant as compared with control group. Groups have the same letter have not significant changes in between them.

Median survival time, percentage increase in lifespan, tumor volume, and weight

Because of carcinoma cell injection, tumor volume reached 3.00±0.28 cm^3, with tumor weight 3.59±0.66 g, which decreased the lifespan of tumor-bearing mice to 20.00±0.18 days whereas treating animals with sabal fraction significantly improved survival parameters. *S. palmetto* extract reduced tumor volume to 0.90±0.03 cm^3 and tumor weight to 0.55±0.07 g, which increased median survival time to 56.00±0.15 days, with increasing lifespan percentage to 180.00% (Table 4). *S. palmetto* extract showed the same ameliorative effect of 5-fluorouracil drug and surpasses it, as tumor-bearing mice treated with Sabal lived more than those treated with 5-fluorouracil.

Liver enzymes activities

Tumor-bearing mice showed highest liver function activity as a result of toxic effect of carcinoma cell injection (AST was 450.00±2.1 U/l and ALT was

110.00±0.1 U/l), whereas the administration of Sabal fractions improved the enzyme activities to record ALT activity as 82.0±0.2 U/l and AST activity as 190.0±0.46. *S. palmetto* fraction showed improving effect like that of standard drug 5-fluorouracil. The positive control of *S. palmetto* extract did not affect liver enzymes of mice with respect to the negative control (data in Table 5).

Discussion

Free radicals and other ROS are considered to be important causative factors in the development of diseases such as neurodegenerative diseases, cancer, and cardiovascular diseases. Different environmental factors and aging elevate the level of free radicals and cells become unable to work efficiently against the free radicals leading to accumulation of radicals and oxidative stress, which results in cellular damage [40].

The antioxidant activity has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging [41]. In healthy biological system, the balance between antioxidants and oxidation process is mostly essential [42].

One of the possible mechanisms of the antioxidative action is the chelation of transition metals. Metal chelating capacity is an important issue as it reduces the concentration of the catalyzing transition metal in lipid peroxidation [43]. It was reported that chelating agents that can make bonds with a metal ion are considered effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion [44]. Transition metal ions can stimulate lipid peroxidation at two ways, participating in the generation of initiating species and accelerating peroxidation, decomposing lipid hydroperoxides into other components which are able to abstract hydrogen, and perpetuating the chain of reaction of lipid peroxidation.

On the contrary, H_2O_2 is able to increase hydroxyl radical in the cells in some cases. Moreover, H_2O_2 leads to transition metal ion-dependent OH radicals-mediated oxidative DNA damage. The H_2O_2 scavenging ability may be attributed to donate electrons to H_2O_2, neutralizes it to water [45].

Active oxygen, in the form of either superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), or singlet oxygen ($^1O_2$), is a product of normal metabolism and attacks biological molecules, leading to cell or tissue injury. $O_2^{\bullet-}$ is also a byproduct of mitochondrial respiration, as well as several other enzymes such as NADH oxidase, monooxygenases, and cyclooxygenases. $O_2^{\bullet-}$ helps in other ROS formation like hydrogen peroxide, hydroxyl radical, and singlet oxygen, which stimulate oxidative damage status in lipids, proteins, and DNA [46]. The toxic effect of $O_2^{\bullet-}$ is through its ability to inhibit iron–sulfur bloc-containing enzymes, which are ticklish in a broad variety of metabolic pathways.

S. palmetto leaves or berries alcoholic extract and berries alcoholic fractions, ethyl acetate and butanol, showed antioxidant characteristics with all previous

Table 4 Effect of *S. palmetto* berries alcoholic extract on survival parameters of tumor-bearing mice

Parameters	Groups		
	Tumor-bearing mice	FU-treated mice	*S. palmetto* treated mice
Tumor volume (cm^3)	3.00±0.28	1.52±0.16**	0.90±0.03**
Tumor weight	3.59±0.66	0.73±0.15**	0.55±0.07**
MST (days)	20.00±0.18	41.00±0.84**	56.00±0.15**
Increase in lifespan (%)	0.0	105.00±0.45	180.00±0.86

Data are presented as mean±SD of 10 animals; FU, 5-flourouracil, MST, median survival time; One-way Analysis of variance was used for data analysis followed by post-hoc test for multiple comparisons (n=6, $P<0.05$); *Mean significant level as compared with tumor-bearing mice group.

Table 5 Efficacy of *S. palmetto* berries alcoholic extract on liver enzymes of tumor-bearing mice in Ehrlich model

Groups	Negative control	Tumor-bearing mice	*S. palmetto* control mice	FU-treated mice	*S. palmetto* treated mice
ALT (U/l)	29.33±1.02[a]	110.00±0.05**	29.00±1.71[a]	90.00±1.51**	82.00±0.21**
AST (U/l)	25.83±1.11[z]	450.00±2.10**	26.31±1.31[z]	192.00±1.05**,[h]	190.00±0.46**,[h]
AST/ALT	0.88±0.21[f]	4.09±1.32**	0.91±0.13[f]	1.92±0.31*	2.31±1.00**

Data are presented as mean±SD of 10 animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FU, 5-flourouracil, one-way analysis of variance was used for data analysis followed by post-hoc test for multiple comparisons (n=6, $P<0.05$); *Mean significant level as compared to control group. Groups have the same letter have not significant changes in between them.

mechanisms. They showed prevention of the chain initiation, binding of transition metal ion catalysts, lipid peroxidation, prevention on continued hydrogen abstraction, reductive capacity, and radical scavenging. Ethyl acetate fraction of berries had the highest effective antioxidant activity compared with other extracts and fractions.

The antioxidant activities of Sabal extracts and fractions may be attributed to capturing of Fe^{2+} or reducing Fe^{3+} and scavenging H_2O_2 which lead to prevention of lipid peroxidation. The ability of Sabal berries crude alcoholic extract to capture Fe^{2+} or reduce Fe^{3+} may be because of the acidic polysaccharides, which contain 10% glucouronic acid, 14% galactouronic acid, 37% galactose, and 12% mannose. Our results were in accordance with those of Ibrahim *et al.* [47] Asker *et al.* [48], and El-Newary *et al.* [49]. They demonstrated that the acidic exopolysaccharides produced from marine organisms are able to inhibit lipid peroxidation and showed antioxidant activities.

Gülçin [50] reported that the compounds with structures containing two or more of the following functional groups: –OH, –SH, –COOH, $–PO_3H_2$, –C=O, $–NR_2$, –S– and –O– in a favorable structure–function configuration can show metal chelation activity. The mentioned active groups can donate an electron or hydrogen atom to eliminate free radicals or reactive species and exhibited antioxidant properties [50]. Moreover, active groups like OH, –SH, –COOH, –C=O, $–NR_2$, –S–, and –O– can compete with oxygen to react with nitric oxide, thereby inhibiting the generation of nitrite. Given the chemical composition of glucuronic acid, galactouronic acid, and other mono-saccharides like galactose and mannose, the major components in the Sabal berries crude extract were found to contain –OH, –COOH, C–H, –C=O, and –O– groups. From the aforementioned presentation together can conclude that the antioxidant activities of Sabal berries crude extract may be attributed to presence of its active groups and its ability to capture Fe^{2+} ion, reduction Fe^{3+}, hydrogen peroxide abstraction, and scavenging SOR, which could be attributed to its lipid peroxidation inhibition ability.

The antioxidant activities of ethyl acetate and butanol fractions of berries extract, which showed the highest antioxidant activities, may be attributed with the polyphenols and flavonoids contents of these fractions. Fractions of ethyl acetate and butanol contain 16.40 and 12.70 polyphenol as mg gallic acid/g extract, respectively, and 5.81 and 4.13 flavonoids as mg quercetin/g extract, respectively.

Polyphenols were documented as antioxidants by various potent mechanisms. The most important antioxidant mechanisms of polyphenols include able to stop the free radical chain reaction, inhibit free radical formation through regulation of enzyme activity, or chelation of metal ions involved in free radical production [51,52]. Polyphenols are able to inhibit oxidases, such as lipoxygenase, cyclooxygenase, myeloperoxidase, NADPH oxidase, and xanthine oxidase, leading to cessation of generation of higher amounts of ROS *in vivo* and organic hydroperoxides. In addition, polyphenols inhibit enzymes indirectly involved in the oxidative processes, such as phospholipase A2, and encourage activities of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase.

Flavonoids carry out their antioxidant activity via the arrangement of active groups at the nuclear structure. In flavonoids, the B ring hydroxyl configuration donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals. Moreover, flavonoids block the enzymes involved in ROS generation, that is, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase. Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation [53].

Most inflammatory diseases are characterized by secretion of PGs which are biosynthesized as a product of cyclooxygenase activity in cells through the consumption of arachidonic acid. Cyclooxygenase genetically includes two isoforms: COX-1 and COX-2. COX-1 mediates the cell vitality processes and is necessary to prevent any deleterious effect on it, whereas COX-2 is produced in cell as a part of inflammatory response to accelerate the production of inflammatory cascade [47].

S. palmetto leaves alcoholic extract, berries alcoholic extract, and the other two fractions, ethyl acetate and butanol, of berries showed anti-inflammatory activity against COX-1 and COX-2 activities, compared with reference drug celecoxib. Alcoholic extract of berries and its fraction ethyl acetate showed the highest activity and inhibited COX-2 by 100% at 500 and 1000 μg/ml. Both of them showed inhibitory effect against COX-2 more than that of celecoxib drug. It is evident from our findings that Sabal materials are more

selective to COX-2 than reference drug, celecoxib, with minimum effect on COX-1.

These anti-inflammatory and antioxidant properties were accompanied with inhibition of nitrite formation. Numerous studies have indicated that NO and PGs participate in inflammatory and nociceptive events. Inhibition of NO and PGs production via the inhibition of COX-2 expression is beneficial in treating inflammatory diseases [54]. All tested materials showed great nitrite formation inhibition percentage, evident by the low IC_{50} of NO scavenging. Many acidic polysaccharides showed an in-vitro and in-vivo anti-inflammatory characteristics [55–57]. Moreover, polyphenols and flavonoids have anti-inflammatory characteristics as that published by Lewis *et al.* [58], Compaore *et al.* [59], and Moschona *et al.* [60].

In addition, the present study clearly demonstrates the antitumor activity of *S. palmetto* berries alcoholic extract against EAC. Any anticancer drug must be a cause of prolongation of animal's lifespan and decrease in WBC count of blood. In current study, results showed an increase in lifespan accompanied by a reduction in WBC count in *S. palmetto* extract-treated tumor-bearing mice. Requirements of tumor cells taken from ascetic fluid, which is the direct nutritional source for tumor growth [61]. *S. palmetto* extract caused significant reduction of the viable EAC cells in animal models. It means that *S. palmetto* extract can reduce the nutritional fluid volume, the source for tumor growth. These results clearly demonstrate the antitumor effect of *S. palmetto* extract against EAC. The major problems of cancer chemotherapy are myelo-suppression and anemia [62]. The anemia in tumor-bearing mice is owing to reduction in RBC and hemoglobin by iron deficiency or hemolytic or myelopathic conditions [63]. However *S. palmetto* extract restored the hemoglobin content, RBC cell count, and WBC cell count to normal values, which indicates the protective effect of *S. palmetto* extract on the hematopoietic system.

Antitumor activity of *S. palmetto* berries extract accompanied with its antioxidants and anti-inflammatory. Our results demonstrated anti-inflammatory activity of *S. palmetto* extract against COX. COX catalyzes the conversion of arachidonic acid to proinflammatory substances such as PG, which can stimulate growth of tumor cells and suppress immune surveillance. Additionally, COX activates carcinogens to take up forms that damage the genetic material [64].

Additionally, the antitumor activity of Sabal berries crude extract may be attributed to its acidic polysaccharide content, which contain 37% galactose, 12% mannose, 10% glucouronic, and 14% galactouronic acids. Many polysaccharides that contain galactose or mannose showed anticancer activities as stated by Zhang *et al.* [65] on polysaccharide from *Inonotus obliquus*, Thinh *et al.* [66] on polysaccharide produced from Brown Alga *Sargassum mcclurei*, Kao *et al.* [67], on polysaccharide isolated from *Ganoderma lucidum*, Kang *et al.* [68] on polysaccharide from *Gracilariopsis lemaneiformis,* and El-Newary *et al.* [49] on acidic polysaccharide produced from marine bacteria. In addition, polysaccharides that contain galactouronic acid and glucouronic acid have antitumor activity as stated by Liu *et al.* [69] on polysaccharides from *Mentha piperita* and Zhang *et al.* [70] on polysaccharides of mushroom *Lentinus edodes.*

Conclusion

S. palmetto berries extract showed potent antioxidant activity with selective anti-inflammatory effect against COX-2 as compared with COX-1 and presented antitumor properties such as immunomodulation, which was determined through ameliorative effect on hematological parameters by increasing animal lifespan, so it can be used as a biological alternative for the well-known Saw palmetto (*S. Serrulata*) plant.

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Conflicts of interest

There are no conflicts of interest.

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