

RESEARCH ARTICLE

Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of *Opuntia stricta* cladodes

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Abstract

Background

The *Opuntia spp.* have been used in traditional medicine for many centuries. It is used in the management of diseases that involves oxidative stress, especially diabetes, obesity and cancer. *Opuntia stricta* (Haw) is one of the relatively unknown species in South Africa where it is regarded more as a weed. Because of this, not much is known about its chemical composition.

Aim

To determine the chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of *Opuntia stricta* cladodes.

Methods

The phytochemical composition of acetone, aqueous and ethanol extract of cladodes of *Opuntia stricta* (Haw), as well as the vitamins A, C and E of its dried weight cladodes and the antioxidant activities, were evaluated using standard *in vitro* methods. The anti-inflammatory and cytotoxic activities were evaluated using cell-based assays. The phytochemical composition and vitamins were determined spectrophotometrically, while the antioxidant activities were determined by DPPH, nitric oxide, hydrogen peroxide scavenging activity and phosphomolybdenum (total) antioxidant activity. Anti-inflammatory activity was determined using RAW 264.7 cells, while cytotoxicity was determined using U937 cells.

Results

The phytochemical composition showed a significant difference in the various extracts. The total phenolics were higher than other phytochemicals in all the extracts used. All the extracts displayed antioxidant activity, while most of the extracts showed anti-inflammatory activity. Only one extract showed cytotoxicity, and it was mild.

Conclusion

The results show that the *Opuntia stricta* is rich in polyphenolic compounds and has good antioxidant activity as well as anti-inflammatory activities.

Abbreviations: DPPH, Alpha, alpha-diphenyl- β -picrylhydrazyl; H₂O₂, Hydrogen peroxide; NO, Nitric oxide; COX-2, Cyclo-oxygenase 2; FBS, Fetal bovine serum; BSA, Bovine serum albumin; DMEM, Dulbecco's Modified Eagle Medium; MTT, 3-(4,5dimethylthiazol-2-yl)-2,5diphenyltetrazoliumbromide; DMSO, Dimethyl sulfoxide; LPS, Lipopolysaccharide; ANOVA, Analysis of variance; CELEX, Celecoxib; AG, Aminoguanidine; ROS, Reactive oxygen species; OS, Oxidative stress.

Introduction

Oxidative stress (OS) occurs as a result of an imbalance between generated reactive metabolites also known as reactive oxygen species (ROS) and the body's antioxidant system. It is a normal physiological condition created to maintain redox homeostasis. However, persistence in the imbalance can cause cellular damage and eventually disease. ROS are known to act on some signalling pathways, modulating physiological responses [1]. ROS are generated through the electron transport chain in the mitochondria, and the cytochrome P450 [2]. Proteins and lipids are some of the major targets for attack, and their modification can lead to some diseases [3]. ROS has been linked to a number of diseases, most of which are chronic diseases. They include atherosclerosis[4], cardiovascular diseases[5], diabetes[6], inflammatory diseases [7], cancer[8] etc. Most of these diseases have a background inflammation, which is chronic in nature and involves the release of ROS. In some cancers, ROS is known to promote cell survival and proliferation [9] as well as play a role in drug resistance [10]. ROS is equally involved in the expression of inflammatory markers [11] some of which play a role in cell proliferation and metastasis[12] as well as mediate immunity [13].

Since ROS are very important in cellular homeostasis and body physiology, regulation of ROS via the body's endogenous antioxidants is a safe means to keep ROS production in check. However when this fails (which happens in diseases), reversing the process through other means becomes very important. Over the years antioxidant supplementation has been used for the prevention and management of ailments caused by oxidative stress[14]. Some of these natural compounds that are polyphenols and vitamins affect many biological processes. They interact with ROS and other inflammatory mediators, modulating their activities to prevent cellular stress [15] which can lead to cellular transformation and eventual cancer [16]. These natural compounds are found in plants and include Vitamins C,E and D, the carotenoids and complex polyphenols. Their mechanism of actions includes inhibition of the catalytic enzymes involved in ROS production, Scavenging of ROS, and upregulation of endogenous antioxidant defence [17]. Vitamins have also been shown to play a role in inhibiting ROS production especially in cancers [18]. For example, vitamin C was reported to induce differentiation and death of acute myeloid leukaemia cells in both *in vitro* and orthotopically transplanted mice[19][20].

The need for powerful antioxidants and anti-inflammatory agents to inhibit the process of cellular transformation have made inroads into plants and herb sources. Phenolic compounds and flavonoids are reported as having excellent antioxidant properties [21].

Opuntia spp. represent one of the most diverse and distributed genera of plants. It is found on all continents except Antarctica[22]. It has its highest degree of diversity in Mexico, where it probably originated, with various degree of domestication observed. It has since been introduced all over the world and can be found in temperate, subtropical and tropical regions. It was introduced into South Africa in the 1700s where it is regarded as a weed and has been actively controlled using biological means. It is a member of the family Cactaceae, subfamily Opuntioideae, tribe Opuntieae. About 1500 species of cactus are in the genus *Opuntia*. The plants (especially *Opuntia ficus indica*) are known as health-promoting foods with their sweet, edible fruits and young cladodes eaten as a vegetable in salads; and also some medicinal properties[23].

Opuntia spp. extracts have been used for several centuries in the management of different ailments which include chronic and inflammatory conditions like diabetes, rheumatism, asthma, hypercholesterolaemia, and hypertension[24]. Recent scientific studies have further increased interests in these plants. Mice fed with methanolic extract of *Opuntia joconostle* seeds showed a significantly lowered plasma LDL cholesterol and triglycerides levels compared to animals fed with placebo [25]. Although it has not been reported to be used as an

antineoplastic agent in traditional medicine, scientific studies carried out showed *Opuntia* has some activity on cancer. Work done by Kim and colleagues revealed that extracts from *Opuntia humifusa* cladodes could cause apoptosis in MCF-7 cells and human colonSW-480 cells [26]. Water partitioned fractions of stem and fruits of *Opuntia humifusa* has also been reported to inhibit the growth of U87MG glioblastoma cells with increased production of reactive oxygen species in the cells [27].

While several species of the family *Opuntiaceae* have been investigated, there is a dearth of information on the biochemical properties of *Opuntia stricta* (phytochemical composition, essential oils, antioxidant, anti-inflammatory, cytotoxic activities). This study aimed to estimate the total phenol, flavonoid, flavonol, proanthocyanidin, tannins, alkaloids, saponins and phytate contents in the water, acetone and ethanol extracts of *Opuntia stricta* cladodes as well as the vitamins A,E and C content and essential oils composition. The study also investigated the antioxidant, anti-inflammatory and the cytotoxic profile of the plant in order to justify its traditional use and add to the body of knowledge.

Opuntia stricta cladodes in this study were discovered to have all the phytochemicals investigated for. It equally showed good antioxidant activities when compared with the standards in the various experiments. It showed comparable anti-inflammatory activity to Celecoxib, a selective COX-2 inhibitor. However its cytotoxic activity was mild against the cell lines used. The essential oils showed it has compounds with antioxidant, anti-inflammatory and cytotoxic activities.

Materials and methods

The cladodes of *Opuntia stricta* were collected from the plant growing within the University of Fort Hare campus in Alice, South Africa. The plant was authenticated by Tony Dolds at the Albany herbarium in Rhodes University, Grahamstown, South Africa. The cladodes were oven-dried at 40°C and pulverized using a milling machine. About 300g of each of the pulverized samples was extracted separately with 1L of each of the solvents, water, ethanol and acetone for 48 h. The extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness under reduced pressure at different temperatures using a rotary evaporator. The filtrate of aqueous extract obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapour Trap, RV T41404, USA). The extracts were stored away in a refrigerator at 4°C.

Phytochemical analyses

Estimation of total phenol content. The total phenol was estimated spectrophotometrically by using the Folin-Ciocalteu assay method[28]. Here 0.5ml of the extract was added to 2.5ml of 10% Folin-Ciocalteu reagent in tubes. It was then vortexed for 30s and allowed to stand for 10 min at 25°C. 2 ml of 7.5% anhydrous sodium carbonate was added to the solution and vortexed again for another 30s. The tubes were incubated in a water bath at 40°C for 30min for colour development, and absorbance read at 765nm using a spectrophotometer. The total phenolic content was then expressed as mg/g gallic acid (GAE/gm) equivalent using the following equation based on the calibration curve:

$$Y = 0.0052x; R^2 = 0.9846$$

Estimation of total flavonoid content. The total flavonoid content was estimated spectrophotometrically by using the aluminium chloride colourimetric assay[28]. The solution was made up of 0.5ml of the plant extract, 2ml of distilled water in a tube and 0.15 ml of 5% sodium nitrite. The solution was left for 5 min at room temperature then 0.15 ml of 10%

aluminium chloride was added it and incubated for another 5 min. After incubation, 1ml of 4% sodium hydroxide was added and the solution made up to 5 ml with distilled water. It was then vortexed and incubated for 15 min to observe a colour change. Absorbance was measured at 420nm. The total flavonoid content was calculated as mg/g quercetin equivalent using the following equation from the calibration curve:

$$Y = 0.0029x; R^2 = 0.997$$

Estimation of total flavonol content. The total flavonol content was estimated using the method described by Wintola and Afolayan [29]. 2 ml of the plant extract was added to 2 ml of 10% aluminium chloride prepared in ethanol. To this 3 ml of 5%, sodium acetate was added and then incubated at 20°C for 2¹/₂ h. The absorbance was measured at 440nm with a spectrophotometer. Total flavonol content was expressed as mg/g of quercetin equivalent derived from the following calibration curve:

$$Y = 0.0107x; R^2 = 0.9928$$

Estimation of proanthocyanidin. The total proanthocyanidins were estimated using the method described by Caceres- Mella *et al.* [30]. 0.5 ml of the prepared plant extract was added to 3 ml of 4% vanillin-methanol. 1.5 ml of hydrochloric acid was then added to the solution and vortexed. The solution was left for 15 min at room temperature. The absorbance was then read at 500 nm using a spectrophotometer. Total proanthocyanidins content was calculated as mg/g of catechin equivalents using the equation derived from the calibration curve:

$$Y = 0.0025x; R^2 = 0.9923$$

Estimation of tannin content. The total tannin content was estimated using the Folin—Ciocalteu method[28]. 7.5 ml of distilled water was added to a tube containing 0.1ml of the plant extract. 0.5 ml of Folin-Ciocalteuphenol reagent and 1 ml of 35% Na₂CO₃ solution was then added. The whole solution was made up to 10 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min. The absorbance was read at 725 nm using a spectrophotometer. A prepared set of standards of gallic acid was prepared in the same manner as the extracts as described earlier. The total tannin content was expressed as mg/g GAE using the following equation derived from the calibration curve:

$$Y = 0.0122x; R^2 = 0.9838$$

Estimation of alkaloids content. The total alkaloid content was estimated using the method described by Unuofin *et al.*[31]. 5 g of the pulverized plant was soaked in 200 mL of 10% acetic acid in ethanol. It was allowed to stand for 4h at room temperature. It was subsequently filtered, and the filtrate was concentrated using a water bath at 55°C to a quarter of its original volume. Concentrated ammonium hydroxide was added in single drops until completion of the precipitation process. The solution was then washed with dilute ammonium hydroxide and filtered again. The residue obtained was first dried and then weighed. The alkaloid content was calculated using the equation:

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100$$

Estimation of saponin. The saponin content was determined according to the method described by Omoruyi *et al.* [32] with some modifications. 5 g of the pulverized plant was added to 20 ml of 20% ethanol and extracted on a shaker for 30 min. The plant sample was heated over a water bath at 55°C for four h. The mixture was filtered, and the residue was re-

extracted again with 20 ml of 20% aqueous ethanol. The filtrate was then reduced to 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel, and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the purification process was repeated. Sixty millilitres (60 ml) of n-butanol was added, and the extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated over a water bath and evaporated to dryness to a constant at 40°C. The saponin content was calculated using the following equation:

$$\% \text{ Saponin content} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Estimation of phytate content. The total phytate content was estimated using the method described by Unuofin *et al.* [31]. 2 g of the pulverized plant was soaked into a conical flask with 50 ml of 2% hydrochloric acid for 3h and afterwards filtered. 25 ml of the filtrate was taken, and 5 ml of 0.3% ammonium thiocyanate solution was added. 53.5 ml of distilled water was also added to achieve the desired acidity. Then 0.05 M of iron III chloride was titrated into it until a reddish brown colour persists for 5 min. Phytate content was calculated as:

$$\text{Phytate}(\%) = \text{titre value} \times 0.00195 \times 1.19 \times 100$$

Antioxidant assays

DPPH radical scavenging assay. For DPPH radical scavenging activity of the plant extracts, the method described by Olajuyigbe and Afolayan [33] was adopted with some modifications. 1 ml of 0.135 mM DPPH in methanol solution was put into tubes with 1ml of various concentrations (0.2–1.0 mg/ml) of the plant extracts, vitamin C, and gallic acid. The mixture was vortexed, then left in the dark at room temperature for 30 min. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Both vitamin C and gallic acid were used as standards. The DPPH radical scavenging activity was calculated from the equation:

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where Abs control was the absorbance of DPPH radical + methanol; Abs sample was the absorbance of DPPH radical + sample extract or standards (Vitamin C and gallic acid).

Nitric oxide scavenging activity. Nitric oxide scavenging activity was determined according to the method described by Boora *et al.* [34] with some modifications. 2 ml of 10 mM Sodium nitroprusside was prepared in 0.5 ml phosphate buffer saline (pH 7.4) and mixed with 0.5 ml of either plant extracts, vitamin C or gallic acid, at various concentrations (0.2–1.0 mg/ml). The mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of Griess reagent (1.0 ml of 0.33% sulfanilic acid reagent prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride) was added to an equal volume of the incubated solution. The mixture was incubated for another 30 min at room temperature, and the absorbance was then measured at 540 nm. The amount of nitric oxide radical inhibited by the extracts was calculated using the following equation:

$$\text{NO radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where Abs control was the absorbance of NO radical + methanol; Abs sample was the absorbance of NO radical + sample extract or standards (Vitamin C and gallic acid).

Hydrogen peroxide radical scavenging assay. For Hydrogen peroxide scavenging activity of the extracts, it was determined using the method described by Oyedemi *et al.* [35]. 4 ml of plant extract, vitamin C or gallic acid was prepared in distilled water at different concentrations (0.2–1.0 mg/ml) and mixed with 0.6 ml of 4 mM Hydrogen peroxide (H₂O₂) solution prepared in phosphate buffer (0.1 M, pH 7.4). The solution was incubated for 10 min at room temperature. The absorbance of the solution was then measured at 230 nm. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

$$H_2O_2 \text{ radical scavenging activity} = \frac{Abs \text{ control} - Abs \text{ sample}}{Abs \text{ control}} \times 100$$

where Abs control was the absorbance of H₂O₂ radical + methanol; Abs sample was the absorbance of the H₂O₂ radical + sample extract or standard (Vitamin C and gallic acid).

Phosphomolybdenum antioxidant assay. The method adopted by Ahmed *et al.* [36] was used to determine the total antioxidant capacity with some modifications. 0.5 ml of plant extracts, vitamin C and gallic acid prepared in varying concentrations (0.1–0.5 mg/ml) were mixed with three ml of distilled water and 1ml of phosphomolybdate reagent in test tubes. The solutions were put in an incubator at 95°C for 90 min. After incubation, the tubes were normalized to room temperature for about 30min. Absorbance was measured at 695 nm. The amount of phosphomolybdenate inhibited by the extract was calculated using the following equation:

$$Phosphomolybdate \text{ antioxidant activity} = \frac{Abs \text{ control} - Abs \text{ sample}}{Abs \text{ control}} \times 100$$

where Abs control was the absorbance of phosphomolybdate reagent + methanol; Abs sample was the absorbance of phosphomolybdate reagent + sample extract or standard (Vitamin C and gallic acid).

Vitamins estimation

Vitamin A estimation. Vitamin A estimation was done by the method described by Onyesife *et al.* [37]. 20ml of petroleum ether was added to 1g of pulverized plant and put on a shaker for about 30mins. The petroleum ether was decanted and evaporated to dryness. 0.2ml of chloroform-acetic anhydride (1:1 v/v) was added to the residue. Later on 2ml of trichloroacetic acid- chloroform (1:1 v/v) was added. The absorbance of the solution was then measured at 620 nm. The vitamin A standard was also prepared in the same way at varying concentrations, and a standard curve plotted. Results were expressed in mg/100g and calculated from the following equation based on the calibration curve:

$$Y = 0.001x + 0.0018; R^2 = 0.9922$$

Vitamin C estimation. Vitamin C estimation was done by the method described by Igwe and Okwu [38]. A 1g of the pulverised plant was put in 20ml of 0.4% oxalic acid. It was then filtered using a Whatman filter paper, and 1ml of the filtrate was mixed with 9ml of indophenol reagent. The absorbance of the solution was measured at 520nm. The vitamin C standard was also prepared in the same way at varying concentrations, and a standard curve plotted. Results were expressed in mg/100g using the following equation based on the calibration curve:

$$Y = 0.67x + 0.0824; R^2 = 0.9714$$

Vitamin E estimation. Vitamin E estimation was done by the method described by Onyeseife *et al.*[37]. 20ml of ethanol was added to 0.5g of the pulverized sample and then left on a shaker for 20mins. It was then filtered. 1ml of the filtrate was then mixed with 1ml of 0.2% of ferric acid in ethanol and 1ml of 0.5% α - α -dipyridine. The solution was made up to 5mls with distilled water. The absorbance of the solution was read at 520 nm. The vitamin E standard was also prepared in the same way at varying concentrations, and a standard curve plotted. Results were expressed in mg/100g using the following equation based on the calibration curve:

$$Y = 0.0086x + 0.0216; R^2 = 0.99$$

Determination of anti-inflammatory activity (Cell line and cell culture)

The RAW 264.7 cells were obtained from the cell culture lab at the Department of Biochemistry, Nelson Mandela University. The RAW 264.7 cells were first suspended in Dulbecco's Modified Eagle Medium (DMEM/low Glucose solution) (Hyclone Laboratories, U.S.A) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 U/mL streptomycin) at 37°C. The cells were stained with trypan blue and the number of viable cells counted using an inverted light microscope (Zeiss) and a Neubauer counting chamber (Hausser Scientific, USA Sigma Cat Z359629). The RAW 264.7 cells were then put into the incubator at 37°C for 24h under a humidified atmosphere of 5% CO₂ to allow for acclimatization.

Quantification of nitric oxide production. After 24h (the cells had adhered to the surface), 100 μ l of the cells each were taken and put in separate wells in a 96 well culture plate. Fifty microliters (50 μ l) of Lipopolysaccharide (LPS), at either 100 μ M or 25 μ M, was added to each well together with 50 μ l of plant extracts (25 and 100 μ g/ml). The 96-well plates were then put back in the incubator at 37 °C for 24hrs. Aliquots (50 μ l) of the cells from each well were removed and added to 50 μ l of Griess reagent (Sigma Cat# 03553) in another 96-well culture plate and incubated at room temperature for 15 min. The absorbance was read at 560nm in a microplate reader (Multiscan MS, Labsystems). All tests were done in triplicate.

Methyl-thiazolyl tetrazolium (MTT) assay. The cytotoxicity assay was carried out on the LPS -induced cells and those with plant extracts and controls using the MTT assay¹³. One hundred microliters (100 μ l) of RAW 264.7 cells were aliquoted into wells of a 96-well culture plate, and One hundred microliters (100 μ l) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide added to each well and incubated at room temperature for 1h. The cell viability was measured at an optical density of 560 nm in a microplate reader (Multiscan MS, Labsystems) All tests were done in triplicate.

Cyclo-oxygenase 2 (COX-2) assay. The RAW 264.7 cells were fixed in formaldehyde at two different concentrations (100 μ M and 25 μ M). The formaldehyde was removed by solubilizing in methanol (50 μ l), and the remaining RAW 264.7 cells kept in the freezer at -20°C for 10 min. The cells were then washed with 50 μ l of 1% bovine serum albumin (BSA) solution, and 50 μ l of 3% BSA and 0.2% Triton X100 added before incubating at room temperature for 45min. Fifty microliters (50 μ l) of antibody was added at 1: 800 dilution and the cells incubated for a further 1h. The cells were then washed twice with PBS before Hoechst solution with 3% BSA was added. The presence of the COX-2 was observed using a fluorescent microscope (ImageXpress XLS Micro [Molecular Devices]). All tests were done in triplicates.

Determination of cytotoxicity. Fresh and dried plant extracts of the various solvents (aqueous, acetone & ethanol) were weighed and dissolved in dimethyl sulfoxide (DMSO). They were then all sonicated for proper solubilization and stored at -20°C. The plant extracts

were then diluted in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and streptomycin medium to its final concentrations.

The U937 and Jurkat cell lines used were obtained from the Department of Biochemistry, Nelson Mandela University, South Africa. The cell lines were maintained in RPMI-1640 (Hyclone Labs, U.S.A) medium supplemented with 5% FBS and penicillin/streptomycin (Biowest, U.S.A). The cells were plated at a density of 2.5×10^4 cells/well in a 96-well plate and cultured overnight in an incubator at 37°C. After 24h the plant extracts were added at various concentrations (12.5, 25, 50, 100, and 200 µg/ml for U937; 25, 50, 100 and 200 µg/ml for Jurkat), then the cells were incubated for another 48h. MTT assay was performed as previously described and the plates incubated for 4h. The absorbance was determined using an ELISA reader (Multiscan MS, Labsystems) at a wavelength of 560nm. All the tests were done in triplicate. Cytosine arabinoside was used as a positive control. Cell viability of the treated cells was determined in reference to the untreated control cells using the following formula:

$$\% \text{ Cell viability} = \frac{\text{Sample Abs}}{\text{Control Abs}} \times 100$$

Gas chromatography-mass spectroscopy (GC-MS) analysis

The GC-MS analysis was performed to determine the chemical make-up of *Opuntia stricta* cladodes. Fresh *Opuntia* cladodes were first extracted of volatile oil using a hydro-distiller in a Clevenger's-type apparatus in accordance with the British Pharmacopeia specifications (1980). The analysis was performed using Agilent 6890 GC coupled to Agilent 5975 MSD with a Zebtron-5MS column (ZB-5MS 30 m x 0.25 mm x 0.25 µm). GC grade helium was used as a carrier gas at a flow rate of 2 mL/min; splitless one µL injections were used. The temperature of the injector was 280°C; the source 280°C, the oven 70°C, the ramp was 15°C/min. to 120°C, then 10°C/min to 180°C, then 20°C/min. to 270°C and held for 3 minutes. The compounds present in the essential oils were identified by matching their spectral mass against the National Institute of Standards and Technology (NIST) 11 database.

Statistical analysis

Data obtained were presented as means ± SD. All experiments were done in triplicates. One way analysis of variance (ANOVA) and the Tukey test were used to determine the differences among the means of the various samples. P values < 0.05 were regarded to be significant.

Results

Phytochemical composition

In this study, the amount of the various phytochemicals investigated varied significantly among the various extracts studied (Fig 1). The total phenolic content of the different solvent extracts of *Opuntia stricta* cladodes showed variable yields in this study. The yields were 101.81mg/g, 82.54mg/g, 54.98mg of GAE equivalent per gram of dried extract of acetone, aqueous and ethanol respectively. The acetone extract of the *Opuntia stricta* cladodes yielded significantly higher phenolic contents than the aqueous and ethanol extract ($P < 0.05$). The aqueous extract was also significantly higher than the ethanol extract ($P < 0.05$). The total flavonoid content of the ethanol extract of *Opuntia stricta* exhibited a higher yield of 57.93mg of quercetin equivalent per gram of dried extract. The acetone and aqueous yields were 20.37, 17.01 mg of quercetin equivalent per gram of dried extract respectively. The ethanol extract showed significantly higher yields than the aqueous or acetone extract ($P < 0.05$). The acetone extract had a higher flavonol content at 16.11mg of quercetin equivalent per gram of dried

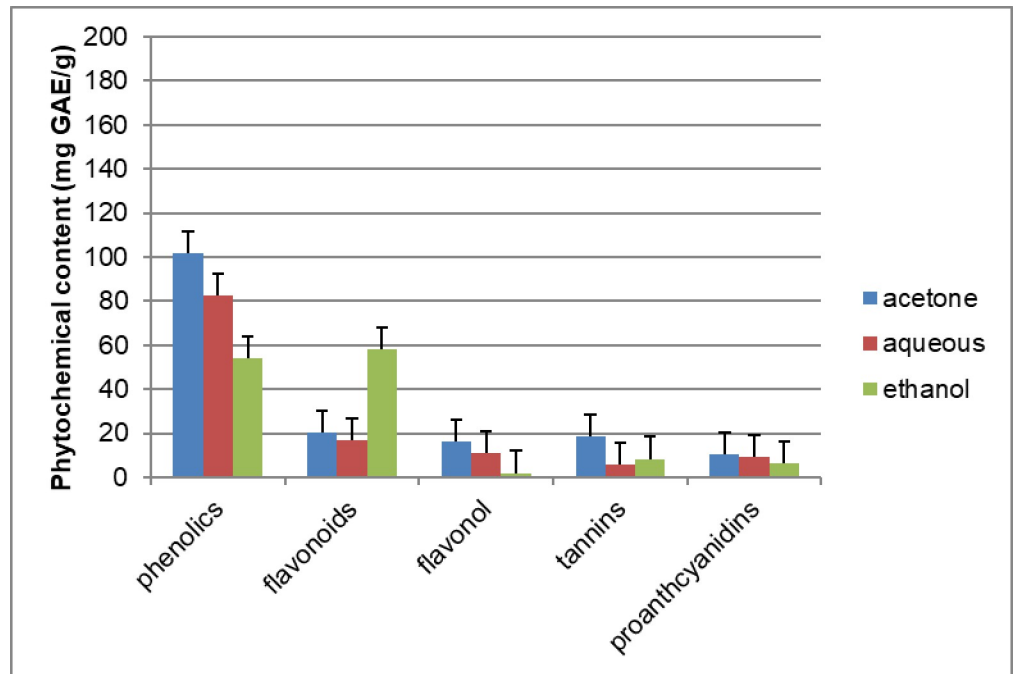


Fig 1. Phytochemical constituents identified in the various extracts of *Opuntia stricta*. Values are expressed as mean ± standard deviation (SD) of three separate determinations (n = 3).

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extract compared to aqueous extract of 10.84mg of quercetin equivalent per gram of dried extract. The ethanol extract was negligible at 1.9mg of quercetin equivalent per gram of dried extract. The acetone and the aqueous extracts showed significantly higher yields when compared to the ethanol extract ($P < 0.05$). The acetone extract of *Opuntia stricta* cladodes gave the highest yield of total proanthocyanidin content at 10.4mg of catechin equivalent per gram of dried extract. The catechin equivalent per gram of dried extract of the aqueous and ethanol extracts were 9.2 and 6.4mg, respectively. The total tannin content of the acetone, aqueous and ethanol extracts were 18.38, 5.84 and 8.32mg of GAE equivalent per gram of dried extract, respectively.

The anti-nutrient content alkaloid, saponin and phytate of the dry macerated cladodes are listed in Table 1. The saponin content was much higher than the phytate and alkaloid.

Vitamin contents of *Opuntia stricta*

Vitamins A, E, and C were present in the dried cladodes of *Opuntia stricta* (Table 2). The highest vitamin content was vitamin A at 711.2 mg/100g of dried extract. The vitamin E content was 231.4 mg /100g of dried extract. The vitamin C content was the least of all the measured vitamins at 2.9mg /100g of dried extract.

Table 1. Anti-nutrient composition of *Opuntia stricta* cladodes.

Bioactive compound	Content (%)
Saponin	93.8 ± 3.43
Alkaloid	0.32 ± 0.02
Phytate	0.37 ± 0

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Table 2. Vitamin content of *Opuntia stricta* cladodes (dried weight).

Vitamin	Mean ± SD (mg/100g)
Vitamin A	711.2 ± 4.2
Vitamin E	231.4 ± 18.6
Vitamin C	2.9 ± 0.08

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DPPH radical scavenging activity

The results of the DPPH scavenging activity of the extracts are as shown in Fig 2. The results showed that ethanol extract had the highest scavenging activity of the extracts at 73.79% ± 0.01. Although none of the extracts in this study had a higher activity than vitamin C and gallic acid used as standards. The acetone, aqueous, ethanol, vitamin C and gallic acid had IC50 values of 0.511, 0.518, 0.510, 0.436 and 0.439mg/, respectively. The study showed that the various extracts have compounds capable of donating protons to the free radicals. This confirms the ability of *Opuntia stricta* to exhibit DPPH free radical scavenging activity.

Nitric oxide scavenging activity

In this study, the extracts and standards (vitamin C and gallic acid) show a concentration-dependent scavenging activity. The ethanol extract showed the highest antioxidant activity at 52.54% ± 0.1. It was higher than the standards used in this study. The IC50 values of the various extracts (ethanol, 0.97mg/ml; acetone, 1.04mg/ml; aqueous, 1.12mg/ml) were comparable to vitamin C (1.18mg/ml) and gallic acid (1.18mg/ml). The result of the nitric oxide scavenging activity is depicted in Fig 3.

Hydrogen peroxide scavenging activity

In this study, the extracts and standards (vitamin C and gallic acid) show a concentration-dependent scavenging activity. The result showed that the aqueous extract had the highest

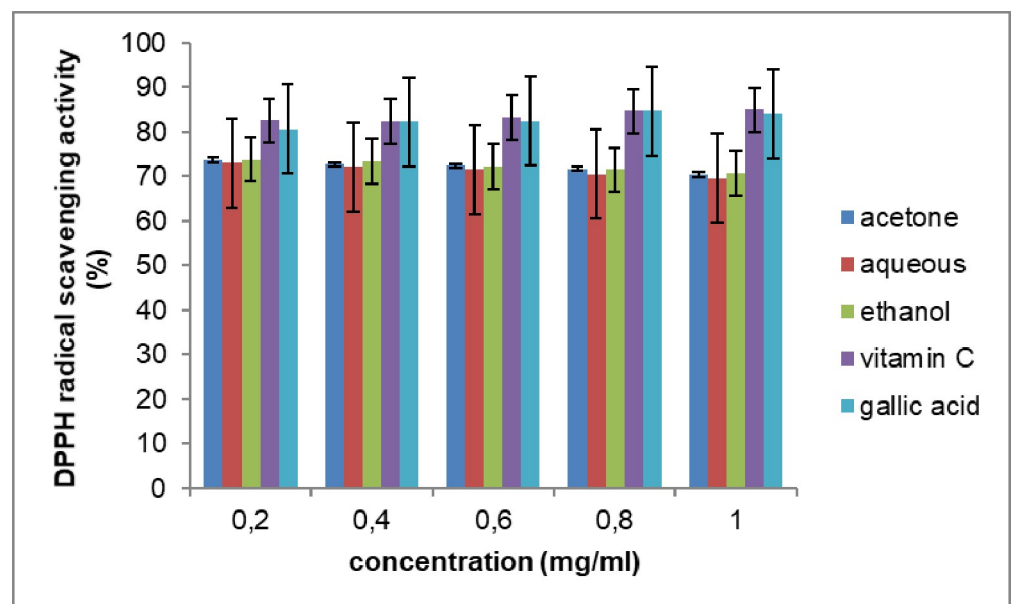


Fig 2. DPPH radical scavenging activity of the extracts of *Opuntia stricta* cladodes. The values represent mean ± S. D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g002>

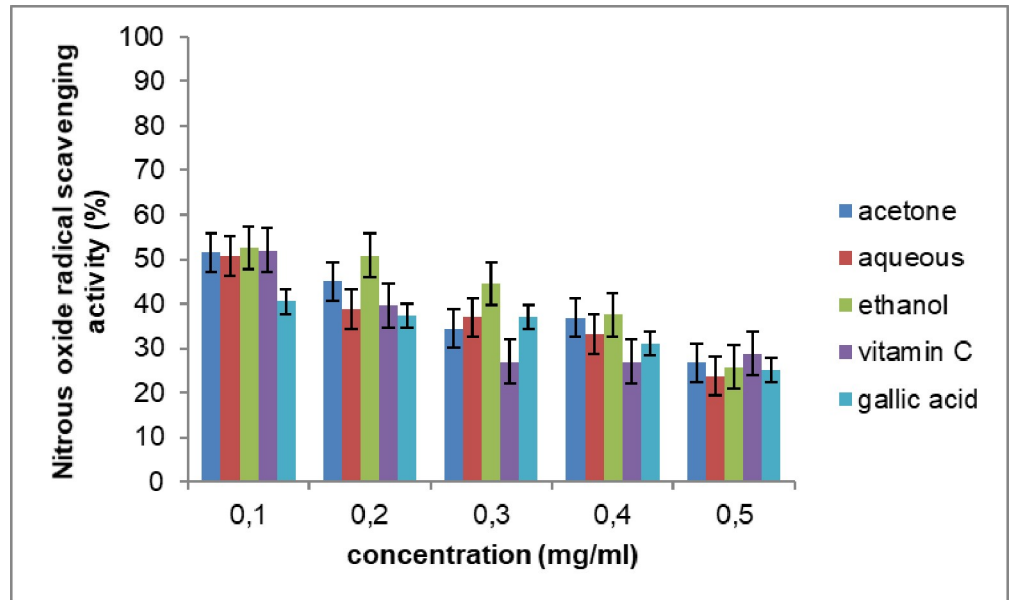


Fig 3. Nitric oxide radical scavenging activity of the extracts of *Opuntia stricta* cladodes. The values represent mean \pm S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g003>

scavenging activity at $98.63\% \pm 0.01$. It was equally higher than the standards used. All the extracts and standards used recorded high inhibitory activities. The acetone, aqueous, ethanol, vitamin C and gallic acid had IC50 values of 0.375, 0.374, 0.379, 0.376 and 0.379mg/ml respectively. These results show that all the extracts used are capable of inhibiting hydrogen peroxide radical. Fig 4 shows the results of hydrogen peroxide scavenging activity.

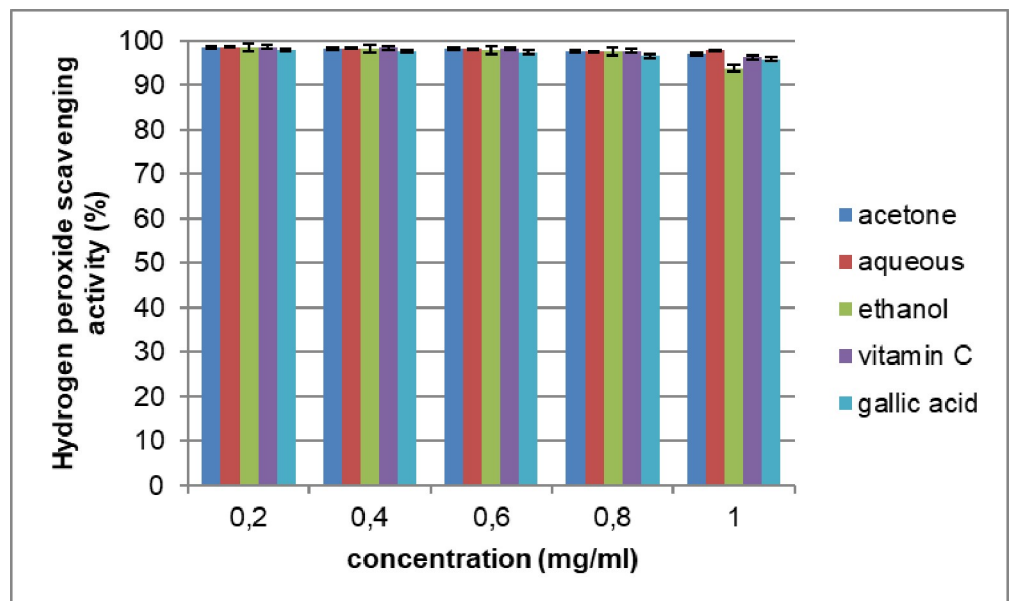


Fig 4. Hydrogen peroxide scavenging activity of the extracts of *Opuntia stricta* cladodes. The values represent mean \pm S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g004>

Phosphomolybdenum (total) scavenging activity

The reducing abilities of the different extracts determined by Phosphomolybdenum (total) scavenging activity were measured spectrophotometrically by their absorbances and summarized in Fig 5. The extracts and standards (vitamin C and gallic acid) show a concentration-dependent scavenging activity. In this study, the aqueous extract of *Opuntia stricta* exhibited the highest total antioxidant capacity at $67.87\% \pm 0.004$. The acetone and the ethanol extracts had activities of $66.15\% \pm 0.006$ and $65.97\% \pm 0.001$ respectively. The scavenging activity of the three extracts was not significantly different and was higher than that for vitamin C and gallic acid. The IC₅₀ values of 0.297, 0.3158, 0.2959, 0.2961, 0.2952 mg/ml were recorded for the acetone, aqueous, ethanol, vitamin C and gallic acid respectively. This study confirms the antioxidant capacity of *Opuntia stricta* cladodes, and its ability to mop up free radicals.

GC-MS analysis of the essential oils

The GC-MS analysis was carried out in order to discover any antioxidant, anti-inflammatory or cytotoxic compound in the essential oil (Fig 6). 46 compounds were identified in the essential oils of *Opuntia stricta*. They include beta-copaene (7.54%), octasiloxane 1,1,3,3,5,5,9,9,11,11,13,13,15,15-hexadecamethyl- (4.75%), Cyclotrisiloxane, hexamethyl- (3.83%), alpha-pinene (3.12%), alpha-Ionone (2.45%), and 5-Methyl-2-trimethylsilyloxy-acetophenone (2.33%). The composition of compounds in the essential oil consisted of monoterpenes, cyclic terpenes, sesquiterpenes, fatty acids, phenols, alcohols, and aromatic compounds. Some of these compounds are known to exhibit some biochemical activity. Beta-ionone and terpinolene, two of the compounds found in the essential oil of *Opuntia stricta* are known to exhibit antioxidant, anti-inflammatory and cytotoxic activities[39][40]. The various compounds found in the essential oils of the plant are listed in Table 3.

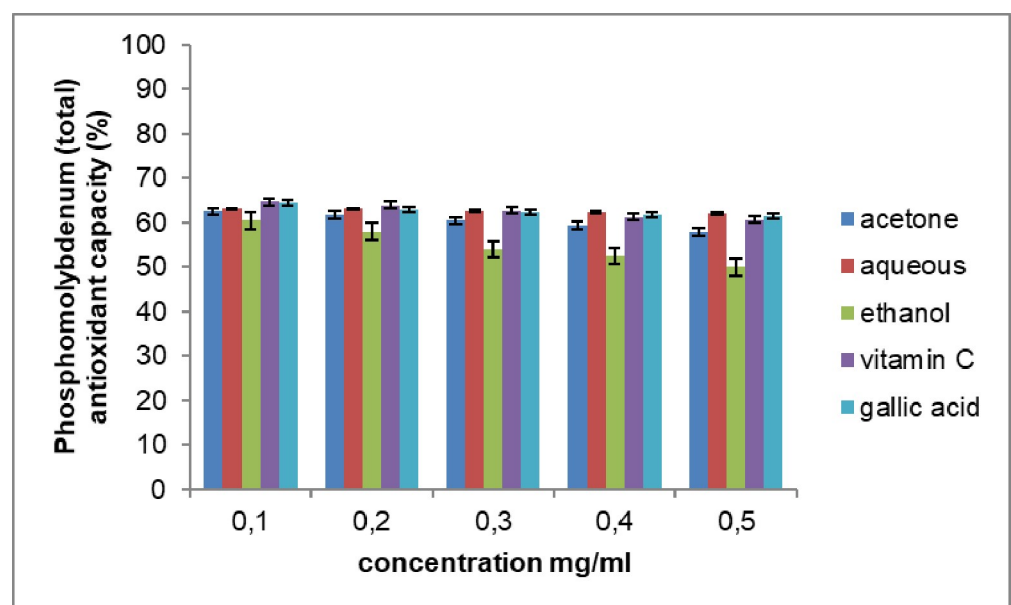


Fig 5. Phosphomolybdenum (total) antioxidant activity of the extracts of *Opuntia stricta* cladodes. The values represent mean \pm S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g005>

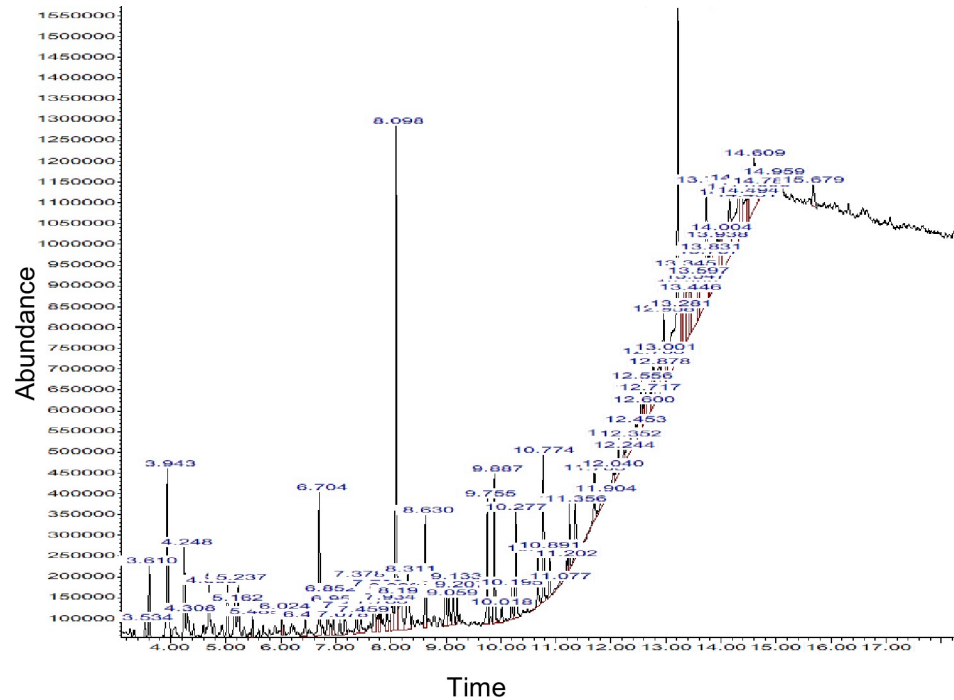


Fig 6. GC-MS chromatogram of essential oil of *Opuntia stricta*.

<https://doi.org/10.1371/journal.pone.0209682.g006>

Effect of *Opuntia stricta* extracts on macrophage toxicity

The RAW 264.7 cells were incubated with the plant extracts in two concentrations: 100µM and 25µM. Cell viability was measured by MTT assay as previously described. From the study, the *Opuntia* extracts had mild cytotoxic effects on the RAW 264.7 cells especially at 100 µM (Fig 7). These results proved that activities of *Opuntia* extracts were not as a result of a reduction in cell viability.

For the COX-2 assay, the numbers of cells that are viable were determined by the Hoechst staining of the nucleic acids as previously described. The *Opuntia* extracts had mild cytotoxic effects on the RAW 264.7 cells, and which occurred mainly at a concentration of 100 µM (Fig 8). The results also showed that the activity of *Opuntia* extracts was not as a result of a reduction in cell viability.

Effect of *Opuntia stricta* extracts on LPS- induced nitrous oxide production

The various levels of nitrous oxide released were measured by an ELISA reader. When LPS alone was added to the RAW 264.7 cells, there was increased production of nitrous oxide as compared to the addition of the plant extracts and the controls. (Fig 9).

The level of nitrous oxide was significantly decreased in the plant extract groups and the controls when compared to LPS-induced only cells ($p < 0.05$). The level of nitrous oxide was however slightly higher in the plant extracts compared to the control group.

Effect of *Opuntia stricta* extracts on COX-2 production

The treatment of the RAW 264.7 cells with *Opuntia stricta* extracts caused a decrease in COX-2 expression in some of the wells (Fig 10). The acetone fresh and the aqueous fresh showed a

Table 3. Chemical composition of *O. stricta* essential oil determined by GC-MS.

S/N	RT (mins)	Compounds	Peak (%)	Structure (ChemSpider Link Address)	Molecular formula	Function	MW (Da)	Hit quality
1	3.534	Dodecamethylcyclohexasiloxane	0.3	http://www.chemspider.com/Search.aspx?rid=497f293c-cce4-45e3-a89e-f37191f1fa83&seq=20	C ₁₂ H ₃₆ O ₆ Si ₆		444.924	46
2	3.613	Heptanal	1.23	http://www.chemspider.com/Search.aspx?rid=b745fc96-2f9b-44a1-8c0e-c14fd07a0193&seq=20	C ₇ H ₁₄ O		114.186	97
3	3.613	Hexanal, 3-methyl-	1.23	http://www.chemspider.com/Search.aspx?rid=ef463d21-63b9-44a0-9283-71022c48f8eb&seq=20	C ₇ H ₁₄ O		114.186	37
4	3.613	Formamide, N,N-dimethyl-	3.12	http://www.chemspider.com/Search.aspx?rid=84c2fdd6-d38b-4079-b6d2-512fc9c6c043&seq=20	C ₃ H ₇ NO		73.094	43
5	3.943	alpha-Pinene	1.46	http://www.chemspider.com/Search.aspx?rid=f238c795-72db-4343-a601-8a1818e8afb9&seq=20	C ₁₀ H ₁₆	Antineoplastic, anti-inflammatory, antioxidant [41][42]	136.234	95
6	4.242	beta-Phellandrene	0.92	http://www.chemspider.com/Search.aspx?rid=84d8f020-b4da-42fe-afc0-c5ae0a27665e&seq=20	C ₁₀ H ₁₆	Antineoplastic, anti-inflammatory, antioxidant [43]	136.234	91
7	4.695	alpha ocimene	0.92	http://www.chemspider.com/Search.aspx?rid=1a45cb0c-56b8-4ada-98a4-3fe8049fb11f&seq=20	C ₁₀ H ₁₆	Anti-inflammatory[44]	136.234	55
8	4.695	sylvestrene	0.95	http://www.chemspider.com/Search.aspx?rid=49a53514-6df3-41ed-aab9-75f92423eaba&seq=20	C ₁₀ H ₁₆		136.234	60
9	5.034	Linalool oxide	1.05	http://www.chemspider.com/Search.aspx?rid=31eef633-5312-41f9-b38d-7f2bfaa9dcc1&seq=20	C ₁₀ H ₁₈ O ₂	Antineoplastic, anti-inflammatory, antioxidant[45][46]	170.249	59
10	5.237	Nonanal	1.05	http://www.chemspider.com/Search.aspx?rid=90308de3-f4ee-4b83-96e3-5a9b156a9c7d&seq=20	C ₉ H ₁₈ O		142.239	68
11	5.237	Cycloheptane	0.36	http://www.chemspider.com/Search.aspx?rid=91248521-ecfc-4a11-905c-0b51102f12ef&seq=20	C ₇ H ₁₄		98.186	47
12	6.024	N-Nitroso-di-n-octylamine	2.45	http://www.chemspider.com/Search.aspx?rid=59ef48c4-c7d5-4633-8737-bae3be493ec8&seq=20	C ₁₆ H ₃₄ N ₂ O		270.454	27
13	6.704	Terpinolene	2.45	http://www.chemspider.com/Search.aspx?rid=cfe99125-e636-41e6-b8cb-06d15c9d2888&seq=20	C ₁₀ H ₁₆	Antineoplastic, anti-inflammatory, antioxidant[47][48]	136.234	70
14	6.704	beta-Ionone	2.45	http://www.chemspider.com/Search.aspx?rid=6cd64782-03e4-407d-9b08-a00b092b4b45&seq=20	C ₁₃ H ₂₀ O	Antineoplastic, anti-inflammatory, antioxidant[49][50]	192.297	70
15	6.704	alpha-Ionone	0.83	http://www.chemspider.com/Search.aspx?rid=2ad01621-527a-4f6f-90d7-923007c087cd&seq=20	C ₁₃ H ₂₀ O		192.297	70
16	6.852	Theaspirane	0.52	http://www.chemspider.com/Search.aspx?rid=59ac34ee-ab5d-453e-80ba-664fa715f931&seq=20	C ₁₃ H ₂₂ O	Anti-inflammatory, antioxidant[51]	194.313	58
17	6.956	Trans-decalin	0.52	http://www.chemspider.com/Search.aspx?rid=a0ea4e92-5d47-46c2-af2e-4c38d5526956&seq=20	C ₁₀ H ₁₈		138.250	50
18	6.956	Gephyrotoxin 181b	0.88	http://www.chemspider.com/Search.aspx?rid=134bd5ec-8d71-44e9-b9a6-48baca292695&seq=20	C ₁₂ H ₂₃ N		181.318	50

(Continued)

Table 3. (Continued)

S/N	RT (mins)	Compounds	Peak (%)	Structure (ChemSpider Link Address)	Molecular formula	Function	MW (Da)	Hit quality
19	7.378	Octadecane, 1-iodo-	7.54	http://www.chemspider.com/Search.aspx?rid=2f126460-54fc-4fcf-8031-c08c5562161d&seq=20	C ₁₈ H ₃₇ I		380.391	25
20	8.098	beta-copaene	7.54	http://www.chemspider.com/Search.aspx?rid=f4e46c4e-552d-45c4-8031-be58bc9dfe5e&seq=20	C ₁₅ H ₂₄	Antineoplastic, antioxidant[52][53]	204.351	93
21	8.098	Gemacrene D	1.5	http://www.chemspider.com/Search.aspx?rid=de69d2a8-b6a1-4586-83bf-de083aa67166&seq=20	C ₁₅ H ₂₄	Antineoplastic, anti-inflammatory[54]	204.351	96
22	8.311	Beta Cadinene	1.5	http://www.chemspider.com/Search.aspx?rid=9a9e795b-23b7-4353-94ed-c66044c410a8&seq=20	C ₁₅ H ₂₄	Antineoplastic, anti-inflammatory[55]	204.351	95
23	8.311	Delta Amorphene	1.5	http://www.chemspider.com/Search.aspx?rid=2e1b0f9d-66bc-438d-9a11-c5dfbfa4bb2&seq=20	C ₁₅ H ₂₄	Antineoplastic, anti-inflammatory[55]	204.351	91
24	8.63	Hexadecane	1.5	http://www.chemspider.com/Search.aspx?rid=cf8c6e05-9e1e-4f88-8d0a-da9d220d682f&seq=20	C ₁₆ H ₃₄		226.441	99
25	8.63	Tetradecane	1.5	http://www.chemspider.com/Search.aspx?rid=db7d9dcb-0cbc-4ac8-a5f6-39439f5fd32c&seq=20	C ₁₄ H ₃₀		198.388	96
26	8.63	Octadecane	0.86	http://www.chemspider.com/Search.aspx?rid=3afd06b-0a6d-426a-94fc-7f57030273d4&seq=20	C ₁₈ H ₃₈	Antineoplastic analogue [56]	254.494	94
27	8.993	Ledol	0.86	http://www.chemspider.com/Search.aspx?rid=a95ef13f-2597-4cb8-ac0e-cb1c06b2762c&seq=20	C ₁₅ H ₂₆ O	Anti-inflammatory, antioxidant[57]	222.366	42
28	8.993	Guaia-3,9-diene	0.86	http://www.chemspider.com/Search.aspx?rid=5508bd39-9781-47a0-82d0-f1776e9e42bb&seq=20	C ₁₅ H ₂₄		204.351	38
29	8.993	(-)-Globulol	0.68	http://www.chemspider.com/Search.aspx?rid=76b9c9cb-2f23-4140-b24f-06e03bcf3a7&seq=20	C ₁₅ H ₂₆ O	Anti-inflammatory, antioxidant[58]	222.366	30
30	9.133	alpha-Cadinol	0.68	http://www.chemspider.com/Search.aspx?rid=12364970-0b3e-4548-bcab-3e1e03659c48&seq=20	C ₁₅ H ₂₆ O	Antioxidant[59]	222.366	94
31	9.133	Alloisolongifolene	0.38	http://www.chemspider.com/Search.aspx?rid=261a2d49-03f8-4617-a667-099d7a6522d2&seq=20	C ₁₅ H ₂₄		204.351	46
32	9.207	Heptadecane	1.74	http://www.chemspider.com/Search.aspx?rid=8685f550-c262-41ff-a429-0280ed4b2c39&seq=20	C ₁₇ H ₃₆	Anti-inflammatory, antioxidant[60]	240.468	95
33	9.755	Heneicosane	1.96	http://www.chemspider.com/Search.aspx?rid=dee77e36-a923-4641-b0c3-f69acc526437&seq=20	C ₂₁ H ₄₄	Anti-inflammatory, antioxidant[61]	296.574	94
34	9.887	Isopropyl myristate	1.96	http://www.chemspider.com/Search.aspx?rid=c8ce7673-6f53-4573-8201-db6f22d4b1d6&seq=20	C ₁₇ H ₃₄ O ₂	Anti-inflammatory, antioxidant[61]	270.451	99
35	9.887	Palmitic acid	1.47	http://www.chemspider.com/Search.aspx?rid=a457ff5e-e9e6-4cb8-8240-399999b96f04&seq=20	C ₁₆ H ₃₂ O ₂		256.424	25
36	10.277	Nonadecane	1.47	http://www.chemspider.com/Search.aspx?rid=07227ed7-8df4-43e7-896f-015b0c505c34&seq=20	C ₁₉ H ₄₀	Anti-inflammatory, antioxidant[61]	268.521	98

(Continued)

Table 3. (Continued)

S/N	RT (mins)	Compounds	Peak (%)	Structure (ChemSpider Link Address)	Molecular formula	Function	MW (Da)	Hit quality
37	10.277	Eicosane	0.72	http://www.chemspider.com/Search.aspx?rid=d10b5c49-1ffe-4976-942e-0458389e99d6&seq=20	C ₂₀ H ₄₂		282.547	96
38	10.678	Dibutyl phthalate	0.63	http://www.chemspider.com/Search.aspx?rid=9b254837-50a3-44dc-9359-a85b0645108e&seq=20	C ₁₆ H ₂₂ O ₄		278.344	92
39	10.891	Isopropyl palmitate	1.16	http://www.chemspider.com/Search.aspx?rid=58dd7f08-c63b-4406-9a00-10a4019b4c36&seq=20	C ₁₉ H ₃₈ O ₂	Anti-inflammatory, antioxidant[62]	298.504	46
40	11.356	Eicosane, 9-octyl-	0.93	http://www.chemspider.com/Search.aspx?rid=45e400f2-052d-4594-a1aa-4d8baf013d87&seq=20	C ₂₈ H ₅₈		394.760	45
41	12.137	Hexamethylcyclotrisiloxane	0.72	http://www.chemspider.com/Search.aspx?rid=14038be8-653b-4c61-b3d9-2ee393afbeff&seq=20	C ₆ H ₁₈ O ₃ Si ₃		222.462	43
42	12.717	2-Ethylacridine	9.14	http://www.chemspider.com/Search.aspx?rid=a2f71291-8f88-4aaf-ad0f-0e97fa30b993&seq=20	C ₁₅ H ₁₃ N		207.270	43
43	13.219	Diisooctyl phthalate	9.14	http://www.chemspider.com/Search.aspx?rid=a9059437-b847-4aba-b3d5-c12ac069018f&seq=20	C ₂₄ H ₃₈ O ₄		390.556	90
44	13.219	Bis(2-ethylhexyl) phthalate	2.33	http://www.chemspider.com/Search.aspx?rid=3dafedd2-7b2a-4f6b-be05-b86c664a6fc0&seq=20	C ₂₄ H ₃₈ O ₄	Pro-inflammatory[63]	390.556	60
45	13.547	5-methyl-2-trimethylsilyloxy-acetophenone	7.54	http://www.chemspider.com/Search.aspx?rid=2bd4fa4e-79d2-4fea-9edf-269a263c8792&seq=20	C ₁₂ H ₁₈ O ₂ Si		222.356	38
46	8.098	beta Cubebene		http://www.chemspider.com/Search.aspx?rid=0166a07b-1a8c-447b-aa21-caadb983a5bf&seq=20	C ₁₅ H ₂₄		204.351	94

<https://doi.org/10.1371/journal.pone.0209682.t003>

COX-2 reduction of about 15% each which were comparable to celecoxib, a selective COX-2 inhibitor at 17%.

Cytotoxic effect of *Opuntia stricta* extracts on U937 cell line

The MTT assay carried out to determine the cytotoxic effects of the various *Opuntia stricta* extracts on the U937 cell line showed that after 48hrs none of the extracts displayed cytotoxic effect. However, at concentrations of 100 and 200µg/ml, the acetone dried extract displayed some activities as indicated in Table 4 and Fig 11. The IC₅₀ was 110.1 µg/ml (Fig 12).

Discussion

The phytochemical composition of each extract differs significantly. The polyphenols (phenols, flavonoids, flavonol, proanthocyanidin, tannins) were the major compounds found in the various extracts of *Opuntia stricta*. They are also well reported to be strongly associated with antioxidant capacity[64]. Phenolic compounds are known to scavenge free radicals, neutralizing their ability to cause cellular damage[65]. These ability to prevent cellular oxidative stress is suggested as a means to prevent chronic diseases[66]. Flavonoids have been studied extensively, and are known to have antioxidative, anti-inflammatory and antineoplastic effects [67]. The cladodes of some cactus plants have been shown to produce some high amounts of flavonoids and flavonoid-like compounds, including isoquercetin and nicotiflorin[68].

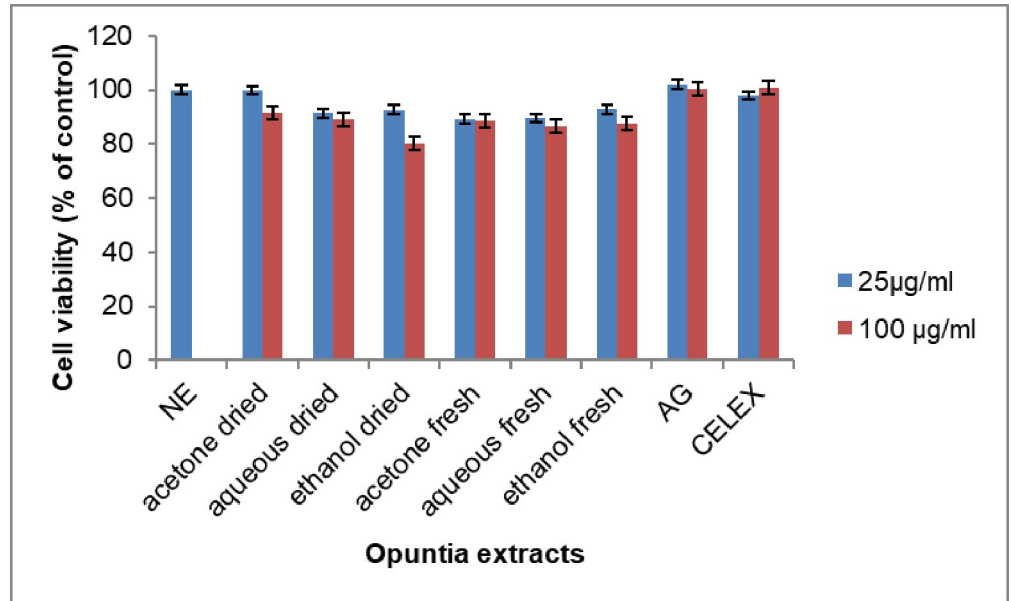


Fig 7. The percentage viable RAW 264.7 cells after 24-hour incubation with either 25 or 100µg/ml LPS and the *Opuntia* extracts. AG = Aminoguanidine. CELEX = Celecoxib. NE = No extract. Each value represents mean ± S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g007>

Nicotiflorin has been shown to have anti-inflammatory and neuroprotective properties against cerebral ischaemia [69], while isoquercetin is currently under clinical investigation as an antithrombotic in certain cancer patients (NCT02195232). The mode of action of polyphenols is through the activation of several signalling pathways including NF-κB, and MAPK p38/JNK which exerts antioxidative and anti-inflammatory effects [70].

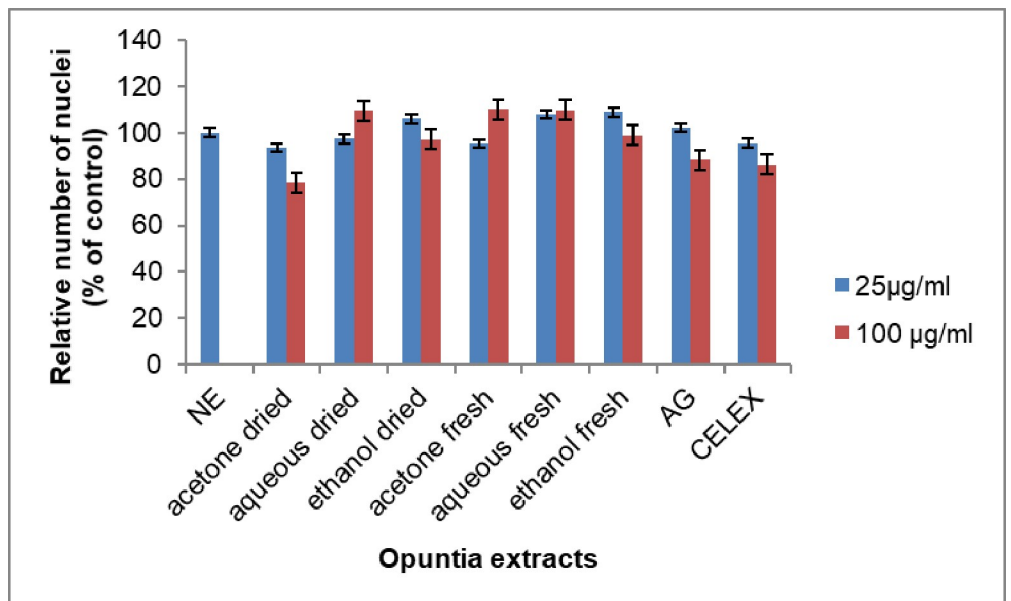


Fig 8. The effect of various *Opuntia stricta* extracts on RAW 264.7 cells density using the Hoechst stain. AG = Aminoguanidine. CELEX = Celecoxib. NE = No extract. Each value represents mean ± S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g008>

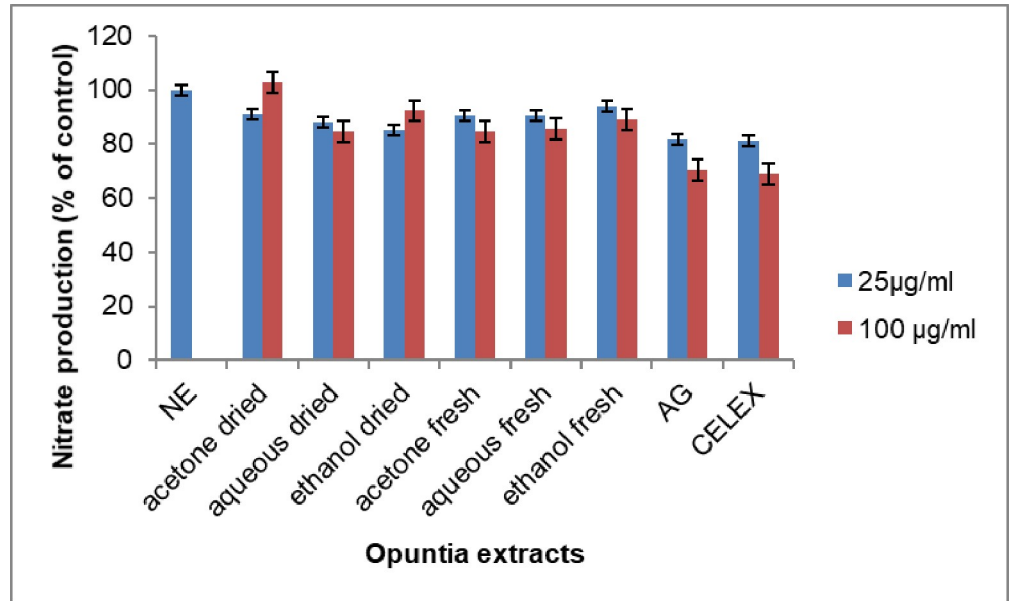


Fig 9. The effect of *Opuntia stricta* extracts on LPS- induced nitrous oxide production in RAW 264.7 cells. NE = No extract. AG = Aminoguanidine. CELEX = Celecoxib. NE = No extract. Values expressed as mean ± S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g009>

Saponin, another type of phytochemicals, are glycosides that contain triterpenoid or spirostane aglycones. There are two main types of saponin, triterpenoid saponins and steroidal saponins. Saponins have been reported to exert different pharmacological actions such as anti-inflammatory [71], immunoregulatory [72] and also antineoplastic [73]. They are also known to display antioxidant properties [74]. The saponin content from the pulverized sample of

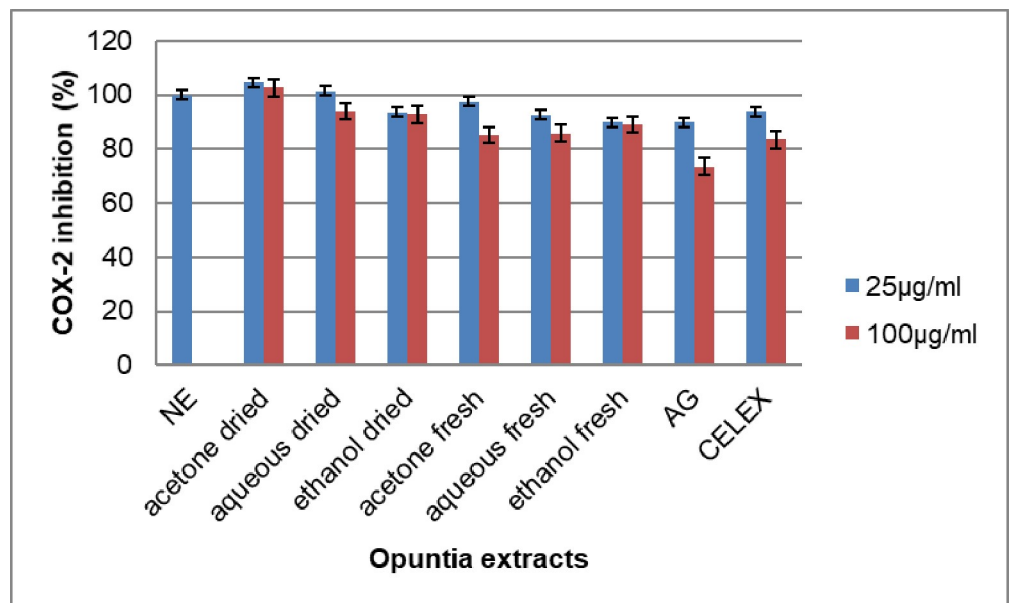


Fig 10. The effect of *Opuntia stricta* extracts on COX-2 expression in RAW 264.7 cells. NE = No extract. AG = Aminoguanidine. CELEX = Celecoxib. NE = No extract. Values expressed as mean ± S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g010>

Table 4. Cytotoxic effect of *Opuntia stricta* cladode extracts on U937 cell line after 48hrs. Values expressed as mean \pm S.D (n = 3).

Conc. ($\mu\text{g/ml}$)	% control	Acetone dried	Aqueous dried	Ethanol dried	Acetone fresh	Aqueous fresh	Ethanol fresh
0	100						
12.5		126.06 \pm 5.5	115.07 \pm 9.2	102.24 \pm 19.6	89.96 \pm 8.8	98.39 \pm 20.4	122.91 \pm 6.9
25		111.13 \pm 6.8	112.69 \pm 22.7	99.86 \pm 12.7	105.1 \pm 2.6	95.98 \pm 8.6	109.25 \pm 5
50		116.96 \pm 2.6	113.22 \pm 12.5	102.42 \pm 17.8	104.47 \pm 4.9	116.97 \pm 16.4	119.79 \pm 13.3
100		46.84 \pm 13.6	139.96 \pm 23.8	109.08 \pm 12.3	118.47 \pm 4.6	103.6 \pm 4.5	118.88 \pm 2.6
200		30.16 \pm 0.8	148.46 \pm 27.7	109.51 \pm 9.15	116.04 \pm 4.9	99.47 \pm 4.5	121.4 \pm 10.7

<https://doi.org/10.1371/journal.pone.0209682.t004>

Opuntia stricta was very high compared to the control, which can easily be explained since carbohydrates are the second highest constituents found in the cladodes of *Opuntia* after water [23]. Since antioxidants play a role in the management of some free radical-related ailments like cancers, the cladodes of *Opuntia stricta* with its rich level of phytochemicals can be used as either dietary or complementary agents.

Vitamins

Vitamins A, E and C were present in the cladodes of *Opuntia stricta*. Vitamins are known to play a role in cellular homeostasis and well-being. They do this by neutralizing ROS development [75] and as such can play a role as antioxidant adjuvants. Vitamins have been used in cancer treatment to mop up free radicals generated during chemotherapy and radiotherapy [76]. The use of high dose retinol supplementation during chemotherapy reduces adverse effects of intestinal malabsorption in children with leukaemia and lymphoma [77]. The Ten-Eleven Translocation-2 (TET2) enzyme activity is known to be enhanced in the presence of vitamin C (TET2 mutation is common in AML), and as such vitamin C has been reported to induce differentiation in leukaemia stem cells [78]. vitamin E is an

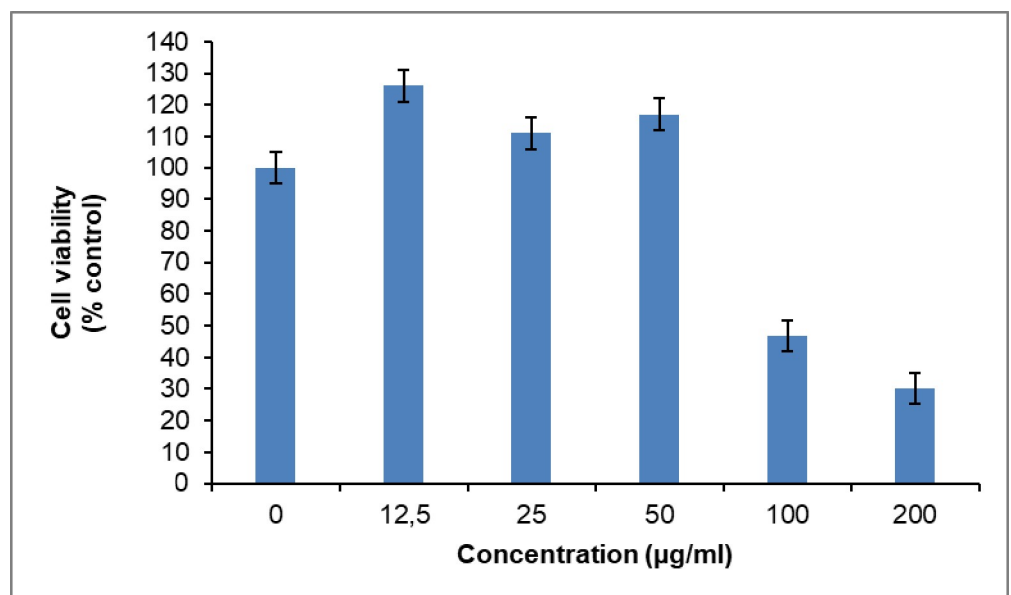


Fig 11. Cell viability of acetone fraction of the dried extract of *Opuntia stricta*. Values expressed as mean \pm S.D (n = 3).

<https://doi.org/10.1371/journal.pone.0209682.g011>

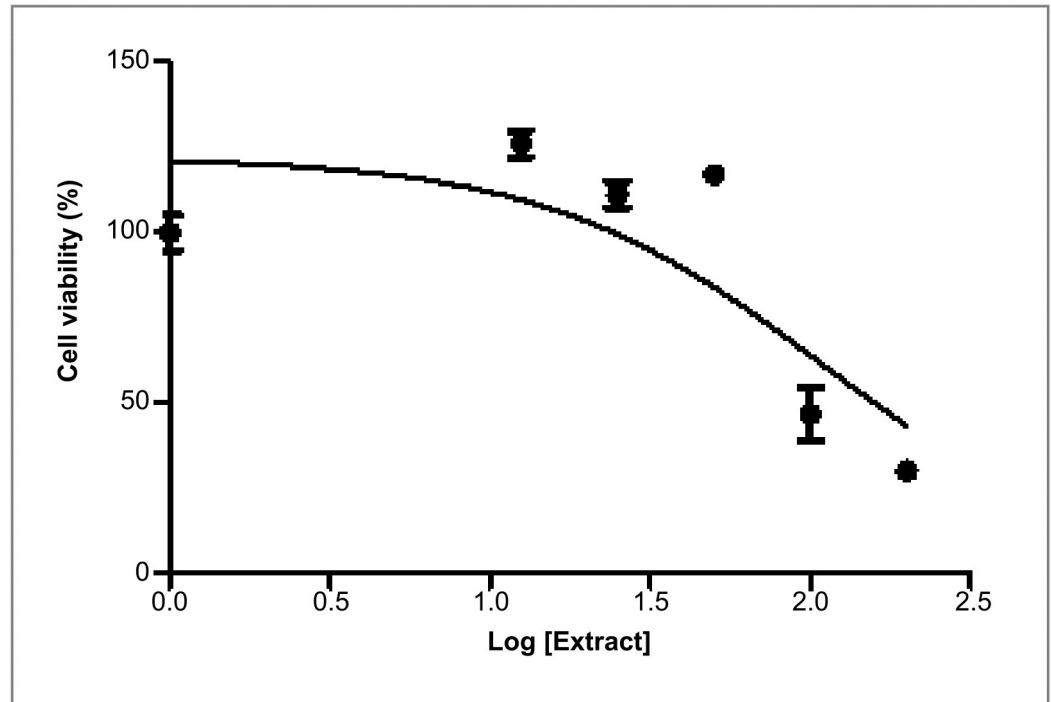


Fig 12. Log(inhibitor) vs response curve of acetone fraction of the dried extract of *Opuntia stricta* on U937 cell line.

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important antioxidant that has been reported to reduce oxidative stress and lipid peroxidation [79]. These vitamins are present in the cladodes of *Opuntia stricta*, and so makes the plant an attractive choice for antioxidant therapy.

Anti-inflammatory

Opuntia stricta extracts caused a decrease in the expression of nitric oxide by lipopolysaccharide-treated RAW 264.7 macrophages cell and COX-2 expression. This proves the anti-inflammatory activity of *Opuntia stricta* extracts. However, while some extracts had an anti-inflammatory effect, a few others caused a slight increased production of nitric oxide and COX-2. The slight increase was more evident in the dry acetone extract (Figs 9 and 10). The anti-inflammatory activity of *Opuntia spp.* is well reported [80][81]. *Opuntia spp.* are also known to induce pro-inflammatory cytokines. Extracts of *Opuntia polyacantha* has been shown to induce production of ROS, nitric oxide and interleukin 6 [82]. The induction of nitric oxide, COX-2 and some inflammatory cytokines are known to play a role in oxidative stress-induced inflammation [83]. Some cancers are known to overexpression COX-2 [84][85]. COX-2 is thus known to mediate cell metastasis [86] as well as mediate immune tolerance through the Indoleamine 2, 3-dioxygenase 1 (IDO1) pathway [87]. This expression of COX-2 is reported to be induced by ROS through activation of NF- κ B and ERK1/2 in macrophages [88]. Thus selective COX-2 inhibitors can have a role to play in cancer management [89].

Some of the compounds found in the essential oil also have good antioxidant and anti-inflammatory properties (Table 3). Compounds like germacrene-D, globulol and caryophyllene also found in *Opuntia stricta* have been shown to have good anti-inflammatory properties [90]. This explains the anti-inflammatory effect shown by *Opuntia stricta*.

Cytotoxicity

While the essential oil of *Opuntia stricta* contains some cytotoxic compounds, the non-cytotoxic effect of the cladodes of *Opuntia stricta* against U937 cell lines may just be that the compounds were not in sufficient amounts. This can also be explained by the low levels of phytochemicals it yielded in this study. This non-cytotoxicity was also observed by Gebresamuel and Gebre-Mariam [91] in their study of *Opuntia stricta* and *Opuntia ficus indica* cladodes. Recently Harrabi *et al.* reported the cytoprotective effect of *Opuntia stricta* cladodes extracts on HepG2 cells[92]. Apart from the level of the phytochemicals, seasonal variation, soil type, and other variables can also affect the level of these compounds in the plant [93]; while the literature quoted higher yields during the summer, the *Opuntia stricta* cladodes in this study were harvested in the winter. In a study done by Alves *et al.* on different *Opuntia spp.* including *Opuntia stricta*, seasonal variations affected the distribution of phytochemicals and nutrients in the plant, and none of the cladodes displayed cytotoxicity against the cell lines used[94].

However, the ethyl acetate fraction of *Opuntia stricta* flower extract was reported to have antineoplastic activity against HepG2 cell line [95]. Betalains, which are water-soluble nitrogenous pigments present in flowers and fruits of *Opuntia spp.*, have also been reported to have good antineoplastic activity against some cancers[96][97]. Betalains from the fruit of *Opuntia ficus indica* was shown to induce apoptosis in chronic myeloid leukaemia, K562 cell line [98] *Opuntia stricta* is known to have the highest total betalain content among all the *Opuntia spp.* studied[23]. So it is possible some other parts of the plants have compounds with antineoplastic activity.

Conclusion

The results show that the cladodes of *Opuntia stricta* are a good source of vitamins, phytochemicals and essential oil with some medicinal benefits. This study reveals that *Opuntia stricta* have antioxidative and anti-inflammatory properties. It is also mildly cytotoxic, which makes it safe to consume as food. These properties make *Opuntia stricta* a good choice as a complementary source to use against diseases that involve oxidative stress. This study is expected to spur more research into *Opuntia stricta* for its therapeutic and palliative uses.

Supporting information

S1 File. UFH Final data Dec 2017 (1). This file contains all the raw data from which the graphs were drawn.
(XLSX)

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