

IN VITRO ANTI-INFLAMMATORY AND FREE RADICAL SCAVENGING ACTIVITIES OF CRUDE SAPONINS EXTRACTED FROM *ALBUCA BRACTEATA* JACQ. BULB.

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## Abstract

**Background:** *Albuca bracteata* is a medicinal plant traditionally used in the management of diabetes mellitus in the Eastern Cape of South Africa. The purpose of this study was to evaluate the antioxidant and anti-inflammatory activities of saponins extracted from the bulb of *Albuca bracteata* and compared with the crude methanolic extract.

**Methods:** *In vitro* antioxidant activity was determined using free radical scavenging assays such as DPPH, ABTS and NO<sub>2</sub>. The anti-inflammatory potential was carried out using inhibition of protein denaturation of egg albumin as a model of anti-inflammatory capacity.

**Results:** Both the crude methanolic extract and saponins showed inhibition of DPPH, ABTS and NO<sub>2</sub> scavenging activity. However, the free radical scavenging activity of isolated saponin compared favourably with Rutin and BHT. The crude methanolic extract showed higher inhibition percentage of protein denaturation compared with the saponins at the concentration investigated.

**Conclusion:** This study indicates that saponin from *Albuca bracteata* bulb possess potent anti-inflammatory activity and is also a good source of natural antioxidant.

**Key words:** anti-inflammatory, *Albuca bracteata*, free radicals, antioxidants

## Introduction

Inflammation is a series of pathological changes associated with local vascular reaction and cellular response of the living tissue to injury. It is a normal protective response to invading microorganisms, irritants and tissue repair (Chandra et al., 2012). Chronic inflammation has become the major health problems of the world. It has therefore become the focus of global scientific research because of its implications in virtually all human diseases (Morales et al., 2014). For instance, a lot of inflammatory mechanisms have been described to induce insulin resistance, decrease insulin secretion and dysfunction of  $\beta$ -cells. (Akash et al., 2013; Uzayisenga et al., 2014). The imbalance of cellular redox homeostasis through excessive production of reactive oxygen species (ROS) contributes to the pathogenesis of several diseases including inflammation (Gupta et al., 2012; Senevirathne et al., 2012; Halliwell, 1997). Although endogenous antioxidants such as SOD, GPx, GSH are more powerful radical scavengers than those from the diets, but under pathological conditions they are overwhelmed by free radicals, therefore, there is need for supplements from external sources.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to manage inflammatory conditions but are with side effects especially gastric irritations which can lead to gastric ulcers (Tripathi, 2008) and with traditional medicine contributing immensely towards the development of modern medicine, it is worthy to note that certain compounds of plant origin can be effective in managing inflammation and oxidative stress with little adverse effect. Many of these compounds and plant extracts have been investigated for their antioxidant actions in several studies. (Francis et al., 2002; Olorunnisola et al., 2011; Oyedemi et al., 2010; Wintola & Afolayan, 2011; Yu et al., 2002). Saponins are a heterogeneous group of naturally occurring active glycosides with triterpenoid or steroid aglycone and complex oligosaccharide moiety (Francis et al., 2002). The sugar and aglycone parts of saponins give them the hydrophilic and lipophilic properties respectively. Saponins are amphiphilic in nature which gives them the surfactant properties and are able to form stable foams in water (Mayer, 1996).

Steroid saponins have been used as raw materials for the production of steroidal hormones, immunological adjuvants while some other types have significant application in food, cosmetics and pharmaceutical industries (Brain et al., 1968; Güçlü-Ustündağ & Mazza, 2007; Hassan et al., 2012).

*Albuca bracteata* Jacq also known as Inqwe beba (Xhosa) is a plant commonly used in the management of diabetes mellitus in the Eastern Cape, South Africa. Earlier studies indicated the presence of alkaloids, saponins, flavanols and flavonoids while the anti-inflammatory potential of aqueous leaf extract of a plant in the same family has been reported (Umapathy et al., 2010). In this study, the *in vitro* anti-inflammatory and antioxidant activities of saponin extracted from *Albuca bracteata* were evaluated.

## Materials and Methods

### Chemicals

2, 2, Diphenyl-2-picrylhydrazyl (DPPH), rutin and BHT were obtained from Sisco Research Laboratories (Pvt. Ltd., Mumbai, India). Diclofenac sodium was obtained from Mylan (Pty) Ltd, South Africa. All other chemicals and reagents used in this study were of analytical grade.

### Plant Material

*Albuca bracteata* (voucher No. ORN14/425) was collected from a forest in Alice (Eastern Cape Province, South Africa). It was identified and authenticated in the department of Botany, University of Fort Hare where voucher specimen was deposited.

### Preparation of Crude Saponin Extract

The bulb of the plant was thinly sliced and oven dried at 40 °C for 72 h, then macerated in Hamilton Beach Commercial Blender type GB27 model HBF 400 - CE. 80 g of the powdered material was extracted in 1 L methanol and placed on an electric shaker for 48 h. The solution was then filtered using Whatman No. 1 filter paper. The filtrate was then concentrated using a rotatory evaporator.

To obtain the crude saponin, the methanolic extract was then fractionalized using the modified method of (Fenwick GR, Price KR, TsuKamoto C, 1992; Igile GO, 1995). Briefly, the methanolic extract was spotted on a TLC plate using hexane : ethylacetate (1:1; 2:1; 1:2) and visualized under UV at 365 nm. Thereafter, it was re-spotted using hexane : ethylacetate : toluene (2:1:2) and visualised under UV at 365 nm. The crude methanolic extract was later loaded into a glass column containing silica gel 60 (0.063 – 0.200 nm) that has been previously washed with hexane : ethylacetate : toluene (2:1:2). The mobile phase was then run through the column and 176 fractions were collected into test tubes in the order hexane : ethylacetate : toluene (2:1:2); hexane : ethylacetate (1:1); ethylacetate (300 ml); acetone (300 ml) and methanol. The fractions were subjected to thin layer chromatography (TLC) on silica gel plates (0.20 mm silica gel) using the solvent system hexane : ethylacetate : toluene (2:1:2). The developed plates were dried at room temperature. Visualization of saponins on developed plates was done by spraying with 0.5 ml anisaldehyde mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid mixed in that order. The TLC plates were then viewed under UV-365 nm. From TLC results fractions A1 – B5; B6 – C11; D1 – E6; E7 – H5; H6 – I5; I6 – P10; P11 were pooled together to get seven fractions and were concentrated.

### Frothing Test

The frothing test was carried out as a confirmatory test of the presence of saponin based on the fact that aqueous solutions of saponins form very stable foams. 1 ml aliquot of each concentrated fractions was shaken with 5 ml of distilled water in a test tube. Stable foams indicated the presence of saponins.

### Determination of ABTS Scavenging Activity

The method of (Re et al., 1999) was used to determine the ABTS scavenging activity. The working solution was prepared by mixing 7 mM of ABTS and 2.4 mM of potassium persulfate in ratio 1:1 in distilled water. The mixture was allowed to react in the dark for 12 h at room temperature. After 12 h, 3 ml of the working solution was further diluted with 150 ml methanol to obtain an absorbance of  $0.706 \pm 0.002$  units at 734 nm using a spectrophotometer. This was adjusted by mixing drop wise of ABTS previously prepared and methanol. 1 ml of the working solution was then added to the test sample of varying concentrations (0.2 – 1.0 mg/ml) and allowed to react in the dark. The absorbance was measured at 734 nm after 7 min. BHT and Rutin were used as standards for ABTS<sup>+</sup> scavenging capacity. The percentage inhibition was calculated as follows:

$$\text{ABTS}^+ \text{ scavenging activity} = \left[ 1 - \left( \frac{\text{Abs sample}}{\text{Abs control}} \right) \right] \times 100$$

Where Abs sample is absorbance of ABTS<sup>+</sup> + sample (or standard)

Abs control is absorbance of ABTS<sup>+</sup> + methanol.

### Determination of DPPH Radical Scavenging Activity

To determine the scavenging activity of DPPH free radical of the samples, the method of (Liyana-Pathiranan & Shahidi, 2005) was used. DPPH in methanol (0.135 mM) was prepared and 1.0 ml of this solution was mixed with 1.0 ml of the extract prepared in methanol containing 0.02 – 0.1 mg of the sample and standards. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. Absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability of the sample extracts to scavenge DPPH radical was calculated from the equation:

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

where Abs control is the absorbance of DPPH radical + methanol

Abs sample is absorbance of DPPH radical + sample/standards.

Rutin and BHT were used as standards.

### Determination Of Nitric Oxide Scavenging Activity

To determine the nitric oxide scavenging activities of the samples, the modified method of (Garraat, 1964) was used. 2 ml of 10 mM sodium nitroprusside previously prepared in 0.5 mM phosphate buffer saline (pH 7.4) was added to 0.5 ml of the samples and standards of different concentrations (0.2 – 1.0 mg/ml) and was then incubated for 2.5 h at 25 °C. 1 ml of the incubated mixture was taken and mixed with 1 ml of Griess reagent (equal volume of 0.33 % sulphanilic acid prepared in 20 % glacial acetic acid and 0.1 % (w/v) naphthylenediamine dichloride) before incubated at room temperature for 30 min. The absorbance was measured at 540 nm and percentage nitric oxide inhibition of the sample was calculated using the equation:

$$\text{NO scavenging activity (\%)} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100$$

Where Abs control is the absorbance of NO radicals;

Abs sample is the absorbance of NO radical + sample or standard.

### Evaluation of in vitro Anti-Inflammatory Activity

The anti-inflammatory activity was carried out by modifying the method of (Chandra et al., 2012). 2.8 ml of phosphate buffer saline (pH 6.4) was added to 0.2 ml of egg albumin. 2 ml of the test solution which contained different concentrations of the test sample or diclofenac was then added. A control was prepared by adding distilled water instead of the test solution. This was then incubated at 37 °C for 5 min (160 L Economy incubator, model 227) before it was heated at 70 °C for 10 min in a water bath. The absorbance was read at 660 nm and the percentage protein inhibition was calculated by the equation:

$$\% \text{ inhibition} = (1 - A/A^0) \times 100$$

Where A = absorbance of test sample and  $A^0$  = absorbance of control

### Statistics

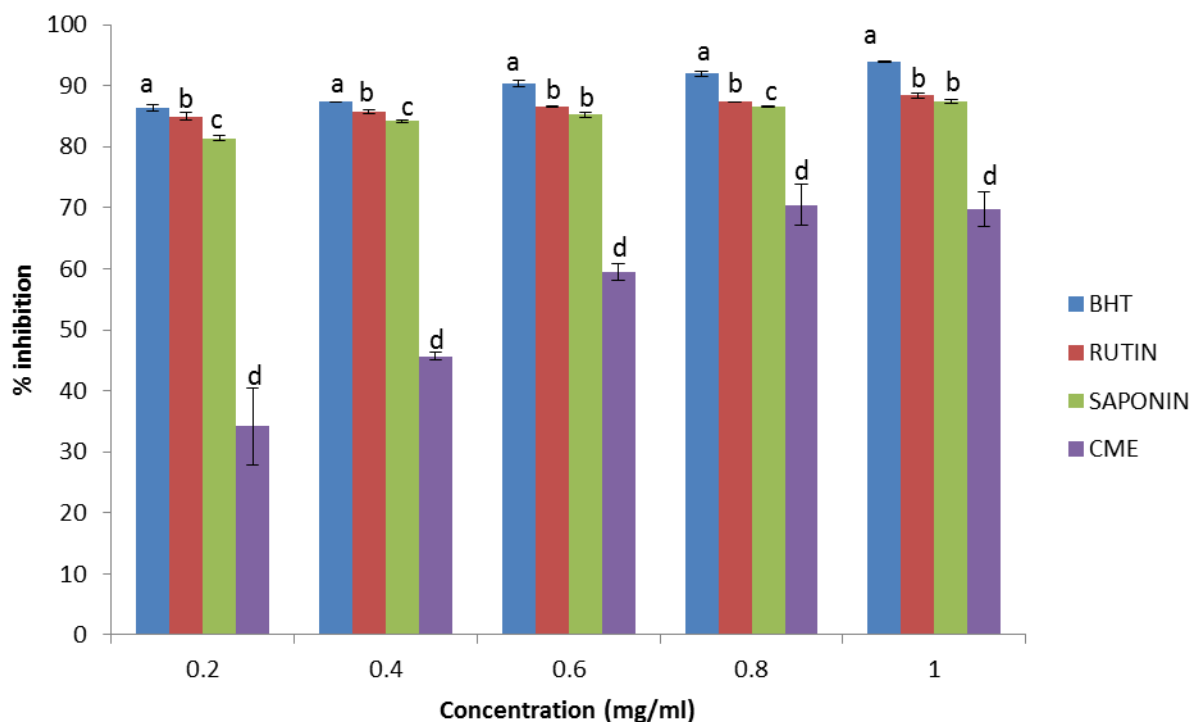
The data obtained from the antioxidant and anti-inflammatory assays were expressed as mean  $\pm$  SD and analyzed using one-way ANOVA followed by Dunnett posthoc t-test for multiple comparisons. P values less than 0.05 ( $P < 0.05$ ) were considered to be statistically significant

## Results

### ABTS Scavenging Activity

The ABTS scavenging activity of the isolated saponin compared favourably with the standards (Figure 1). The crude methanolic extract showed less scavenging activity when compared with the standards and saponin. All the tested samples and the standards showed increase in percentage inhibition in a concentration-dependent manner. The  $IC_{50}$  of both saponin and crude methanolic extract were calculated and found to be lower than the values of the standards used (

Table 1). The  $IC_{50}$  of saponin was higher than the crude methanolic extract but lower than the standards.



**Figure 1:** ABTS scavenging activity of saponin and crude methanolic bulb extract of *Albuca bracteata*. Results are means of triplicates means  $\pm$  SD bars with different letters significantly different ( $P < 0.005$ ), Rutin, BHT = Butylated hydroxytoluene (standards), CME: Crude methanolic extracts

### DPPH Scavenging Activity

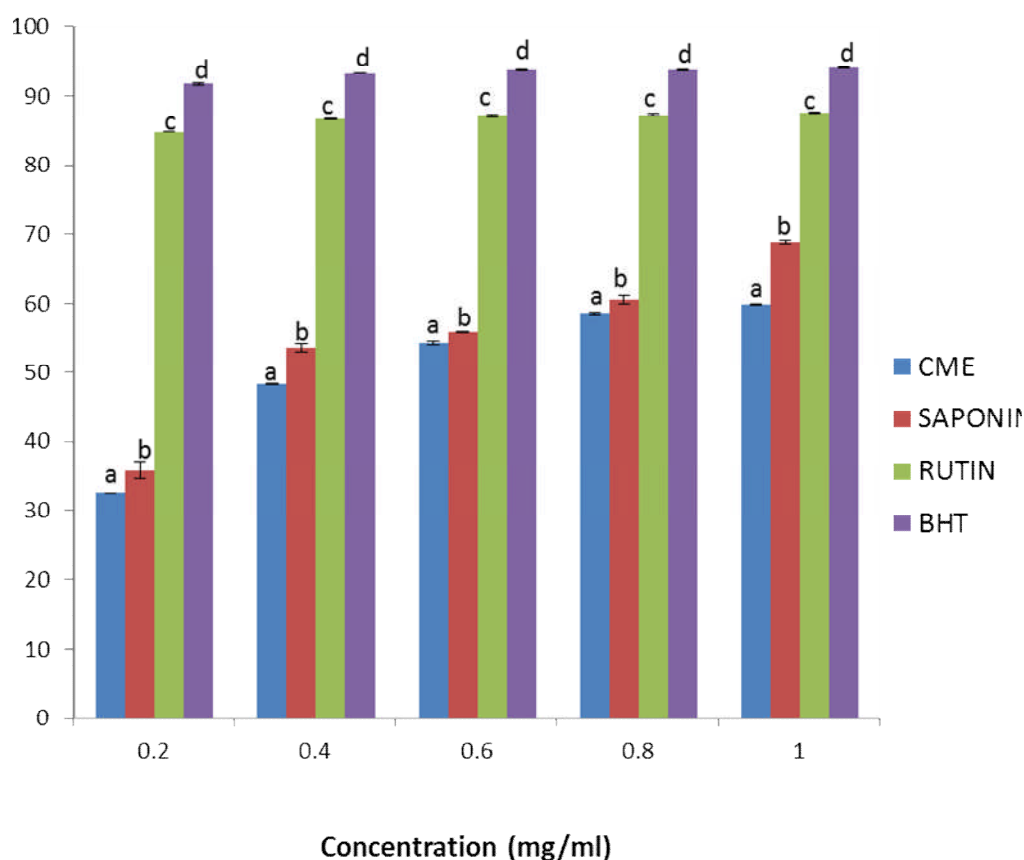
The result of this assay showed that saponin has higher DPPH scavenging activity than the crude methanolic extract in a concentration-dependent manner but is significantly different from each other at 0.4, 0.6 and 1.0 mg/ml only (Figure 2). The scavenging activity of the saponin was significantly lower than that of the standards. The  $IC_{50}$  of the saponin was also observed to be higher than the crude extracts and standards (

Table 1).

**Table 1:** IC<sub>50</sub> Scavenging activity of *Albuca bracteata* bulb extracts and standards

Sample	DPPH		ABTS	
	IC <sub>50</sub>	R <sup>2</sup>	IC <sub>50</sub>	R <sup>2</sup>
<b>Saponin</b>	0.530	90.17	0.526	94.67
<b>CME</b>	0.462	85.43	0.51	92.8
<b>Rutin</b>	0.439	78.19	0.620	99.49
<b>BHT</b>	0.442	76.15	0.617	98.07

IC<sub>50</sub> is defined as the concentration (mg/ml) sufficient to obtain 50% of a maximum scavenging capacity  
R<sup>2</sup>: coefficient of determination; values obtained from regression lines with 95% confidence level.  
CME: Crude methanolic extract



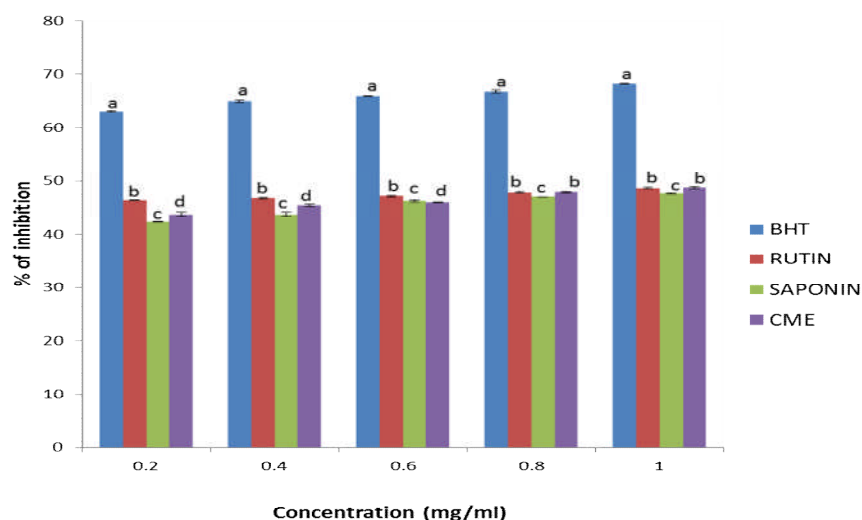
**Figure 2:** DPPH scavenging activity of saponin and methanolic bulb extract of *Albuca bracteata*. Results are means of triplicates a-d means  $\pm$  SD bars with different letters significantly different ( $P < 0.005$ ), Rutin, BHT = Butylated hydroxytoluene (standards), CME: Crude methanolic extracts.

#### Nitric Oxide Scavenging Activity

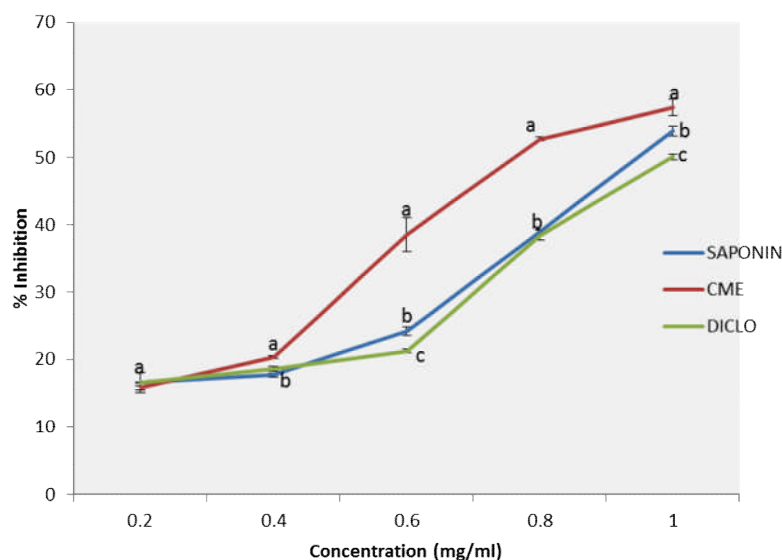
The nitric oxide scavenging assay indicates that both the saponin and crude extracts showed nitric oxide inhibitory activity in a concentration-dependent manner (Figure 3). However, the crude extract has more nitric oxide inhibition activity than the crude saponin extract but are not significantly different from each other.

#### In Vitro Anti-Inflammatory Assay

The *in vitro* anti-inflammatory assay (Figure 4) indicates that crude methanolic extract of *A. bracteata* has higher percentage inhibition of protein denaturation than the saponin in a concentration-dependent manner. The inhibition of saponin compared favourably with that of diclofenac at the concentration investigated. The IC<sub>50</sub> of crude methanolic extract was also lower compared with saponin and diclofenac sodium (Table 2 ) respectively.



**Figure 3:** Nitric oxide scavenging activity of saponin and methanolic bulb extract of *Albuca bracteata*. Results are means of triplicates a-d means  $\pm$  SD bars with different letters significantly different ( $P < 0.005$ ), Rutin, BHT = Butylated hydroxytoluene (standards), CME: Crude methanolic extracts.



**Figure 4:** Anti-inflammatory activity of saponin and methanolic bulb extract of *Albuca bracteata*. Results are means of triplicates a-c means  $\pm$  SD bars with different letters significantly different ( $P < 0.005$ ), DICLO = Diclofenac (standard), CME: Crude methanolic extracts

**Table 2:**  $IC_{50}$  activity of *Albuca bracteata* bulb extracts and standards

Sample	DPPH	
	$IC_{50}$	$R^2$
Saponin	0.704	90.49
CME	0.594	95.89
Diclofenac	70.04	88.01

$IC_{50}$  is defined as the concentration  $[(\mu\text{g/ml})$  for Diclofenac,  $(\text{mg/ml})$  for CME and saponin sufficient to obtain 50% of a maximum inhibition capacity;  $R^2$ : coefficient of determination; values obtained from regression lines with 95% confidence level.

CME: Crude methanolic extract

## Discussion

The antioxidant capacity of medicinal plants have been attributed to the presence of alkaloids, polyphenols and saponins (Rao & Gurfinkel, 2000; Sati et al., 2010). Antioxidant property of many medicinal plants are responsible for their therapeutic potentials in the management of degenerative diseases such as diabetes, cancer, inflammation, atherosclerosis (Elekofehinti et al., 2012). Due to the fact that they are cheap and readily available than synthetic agents, antioxidant capacity of medicinal plants has been well exploited in recent times.

The ABTS and DPPH radical scavenging activities of the extract were similar to the result observed in the earlier studies (Odeyemi et al., 2015 submitted for publication). The free radical scavenging activity of saponin was higher than the methanolic extract, this could be due to inhibitory effect of other compounds present in the crude methanolic extract and purification of the saponin could increase the free radical scavenging activity. The free radical scavenging activity of the plant may be of importance to reduce cellular damages that cause lipid peroxidation and inflammation.

The inhibition of protein denaturation suggests that both the crude methanolic extract and saponin isolated have significant anti-inflammatory activity that might be mediated through the inhibition of the release or synthesis of the agents that produce inflammations. Although the percentage inhibition of crude methanolic extract were higher than the saponin this may be attributed to the presence of another therapeutic agents in the crude methanolic extract. The ability of the extract and saponin to inhibit thermal and hypotonic protein denaturation may contribute to its anti-inflammatory properties similar to the mode of action of NSAIDs (Mizushima, 1964).

Ndebia et al. (2011) suggested that the precise mode of action of saponin could be through the inhibition of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity or cyclooxygenase cascade which blocks the release of vasoactive substances like histamine, serotonin and kinins.

Another modes of actions have been shown to be through the inhibition of COX-2, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Yaun et al., 2006). Because nitric oxide is essential for cellular signalling, it is involved in many physiological and pathological processes including inflammation (Leiper & Nandi, 2011), protection of liver ischemic damage (Yang et al., 2011), carcinogenesis and rheumatoid arthritis (Melchers et al., 2006). Therefore, inhibition of nitric oxide may suggest the mechanism of saponin against inflammation.

Saponin has also been thought to exert its activity through the inhibition of TNF -  $\alpha$ , a cytokine that is also involved in systemic inflammation through the regulation of immune cells, acute phase reaction and induction of apoptotic cell death (Grivennikov & Karin, 2011; Chen et al., 1994). Also, the numerous biological activities of saponins have also be suggested to be linked to their amphiphilic nature which could also explain their ability to intercalate into the plasma membrane resulting in changes in membrane fluidity that in turn may affect membrane function (Hassan et al., 2012).

## Conclusion

The result of this study shows that saponin and methanolic extracts are good source of natural antioxidants and possess free radical scavenging activity. They were also effective inhibiting albumin denaturation induced inflammatory process. This may, however, be the mode of action through which *Albuca bracteata* exerts its anti-diabetic properties as claimed by the traditional healers.

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