

# Phytochemical Screening, Antioxidant, Anti-inflammatory, and Glucose Utilization Activities of Three South African Plants Used Traditionally to Treat Diseases

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## Abstract

Medicinal plants are important in the treatment of human diseases. The medicinal properties of plants are attributed to the presence of secondary plant metabolites. Polyphenols, particularly flavonoids, are phytochemicals that possess antioxidant and anti-inflammatory activity, and modulate pathways involved in obesity and glucose metabolism. In folk medicine, a single plant may be used to treat various diseases owing to the presence of phytochemical varieties in plants. In this study, the acetone leaf extracts of *Curtisia dentata*, *Pittosporum viridiflorum*, and *Portulacaria afra* were investigated for total polyphenol and total flavonoid content as well as for anti-inflammatory, antioxidant, and glucose utilization activities by using standard methods. The *C. dentata* extract exhibited the highest polyphenols ( $125.12 \pm 2.18$  mg/g GAE) and flavonoids ( $27.69 \pm 4.98$  mg/g QE). The *P. viridiflorum* extract exhibited the strongest DPPH radical antioxidant activity ( $IC_{50}$  value =  $12.94 \pm 1.09$   $\mu$ g/ml) and anti-inflammatory activity by inhibiting 5-lipoxygenase activity ( $IC_{50}$  value =  $46.50 \pm 4.54$   $\mu$ g/ml). All extracts enhanced dose-dependent glucose utilization activity of muscle cells and adipocytes. *P. viridiflorum* enhanced the highest glucose utilization of C2C12 muscle cells ( $67.34 \pm 0.51\%$ ), while *C. dentata* enhanced the highest glucose utilization of 3T3-L1 adipocytes ( $63.72 \pm 0.48\%$ ) at the highest concentration of 500  $\mu$ g/ml, with no significant difference ( $p < 0.05$ ). Polyphenols may be responsible for the observed 3T3-L1 cells glucose utilization activity of *C. dentata*. However, the polyphenols alone may not be responsible for the antioxidant, anti-inflammatory, and C2C12 cells glucose utilization activity of *P. viridiflorum*. To our knowledge, this is the first study to demonstrate the glucose utilization potential of the species of medicinal plants.

**Keywords:** Medicinal plants; Phytochemicals; Polyphenols; Type 2 diabetes; Anti-inflammatory; Antioxidant

## Introduction

Medicinal plants play an important role in the treatment of human diseases, particularly in the developing world. In this regard, the World Health Organization [1] also realized that an effective health agenda for developing countries can never be achieved by the use of conventional western medicine alone. Accordingly, WHO advised and urged developing countries to utilize their medicinal plant resources and other traditional medicine systems in order to achieve the objective of primary health care [1,2]. In addition, herbal medicines are used in developed countries of the world. The utilization of herbal medicine in developed countries is believed to be motivated by several factors, including the lower side effects of phytomedicines compared with modern synthetic drugs, the lower cost of phytomedicines compared with modern synthetic drugs as well as the effectiveness of some plant remedies [3].

In South Africa, similar to many other developing countries, medicinal plants and herbal formulations play important roles in the daily health care of the people [4]. The country is rich in floral biodiversity, which is a natural reservoir for the preparation of herbal medicine [5]. It is estimated that more than 4000 plant taxa are used in herbal formulations countrywide [6]. Katerere and Eloff [7] have identified many Southern African species that are used to treat diabetes, their chemistry and the pharmacological evidence for their efficacy.

The medicinal properties of plant remedies have been attributed to the presence of secondary plant metabolites including polyphenols, steroids, alkaloids, terpenoids, and glycosides [8]. Phenolic compounds, particularly flavonoids, are secondary plant metabolites that are able to regulate the pathways involved in adiposity and obesity [9]. They also possess antioxidant [10], anti-inflammatory, and blood glucose lowering properties [11]. Therefore, plants rich in such phytochemicals could be used in the treatment of medical conditions associated with chronic hyperglycemia, oxidative stress, and obesity-induced inflammation, such as type 2 diabetes mellitus.

Type 2 diabetes mellitus is a non-insulin-dependent chronic hyperglycemia resulting from insulin resistance in responsive cells in association with pancreatic  $\beta$ -cell dysfunction [4]. It is a genetic disorder, but overweight and obesity are factors responsible for the development of the disease [12]. Obesity is a condition of excessive adipose tissue accumulation that is characterized by the over-production of free fatty acids, adipokines, and activation of inflammatory signaling pathways [13,14]. Studies have demonstrated that chronic hyperglycemia can induce oxidative stress, provoked by excessive generation of free radicals or by the impairment of endogenous antioxidant defense, and has been implicated in the development of diabetic complication [15]. Therefore, a crude plant extract or an isolated and purified phytochemical agent possessing blood glucose lowering effects, in addition to antioxidant and anti-inflammatory activity, may be an ideal treatment for type 2 diabetes mellitus.

In South Africa, the stem bark extracts of the medicinal plants' species *Curtisia dentata* and *Pittosporum viridiflorum*, as well as the

Plant species (family)	Part used	Uses	Reference
<i>Curtisia dentata</i> (Burm.f.) C.A. Sm(Cornaceae)	Stem bark	Obesity, stomach ailments, fever, diarrhea, cancer, hypertension, constipation, aphrodisiac	Oyedemi <i>et al.</i> [17]
<i>Pittosporum viridiflorum</i> (Pittosporaceae)	Stem bark	Back pain, fever, stomach, chest, and abdominal pains. Inflammatory disorders	Iwalewa <i>et al.</i> [18]
<i>Portulacaria afra</i> Jacq. Portulacaceae	Leaves	Rashes and chronic sores	De Wet <i>et al.</i> [19]

Table 1: Previous reports on traditional uses of selected plants' species

leaf extracts of *Portulacaria afra*, are successfully used as traditional treatment agents for a variety of human ailments including obesity, inflammatory disorders, as well as skin infections, respectively (Table 1). Owing to the varieties of the secondary metabolites of plants, a single plant may be used for the treatment of various diseases in traditional medicine [16]. Currently, there are only limited studies on these three South African medicinal plants' species regarding some aspects of glucose metabolism. There is also limited information on the nature of the secondary plant metabolites in the extracts of these plants' species that may be responsible for this medicinal property. Furthermore, it is not known if there is a relationship between the antioxidant and/or anti-inflammatory properties of the acetone extracts of these plants' species and glucose metabolism. The objective of this study was therefore to investigate the leaf acetone extracts of the *C. dentata*, *P. afra*, and *P. viridiflorum* plants' species for glucose utilization activity, anti-inflammatory, and antioxidant activities as well as to determine the total polyphenol and total flavonoid contents.

## Materials and Methods

### Reagents and chemicals

C2C12 mouse muscle myoblast (CRL-1772) and 3T3-L1 mouse pre-adipocytes fibroblast (CL-173) were purchased from American Type Culture Collection (ATCC) (Manassas, VA). Moreover, Trypan blue (Fluka), dimethyl sulfoxide (DMSO), acetone, methanol, and Whatman No. 1 filter paper were purchased from Merck (South Africa). All the other chemicals were purchased from Sigma (South Africa). The absorbance measurements were read using an Epoch micro plate reader (BioTek).

### Plant material

The leaves of *C. dentata*, *P. afra*, and *P. viridiflorum* were collected from the SANBI Pretoria National botanical garden, and voucher specimens were deposited to the Department of Biology, Sefako Makgatho Health Sciences. The leaves were separated from the stems and dried at room temperature. The dried plant materials were milled to a fine powder in a Kenwood dry mill blender and stored at room temperature, in closed glass bottles in the dark, until extracted.

### Preparation of extracts

The powdered plant material (2 g) was individually extracted with 20 ml of acetone (technical grade – Merck) in polyester centrifuge tubes using a platform shaker (Labotec model 20.2 shaking machine) at room temperature for 30 min [20]. The extracts were centrifuged in a Rotofix 32 A Hettich centrifuge, and the clear supernatant was filtered through

Whatman No. 1 filter paper. This procedure was repeated three times on the same plant material with fresh solvent to ensure that all possible compounds were exhaustively extracted. The filtered extracts of each species were combined into pre-weighed glass vials, and the solvent was left to evaporate at room temperature under a stream of cold air. The extracts obtained were used for the subsequent assays.

## Phytochemical Screening

### Total polyphenol content

The total polyphenol content of the extracts was determined using the 96-well plate method adapted from Zhang *et al.* [21]. The extracts (20  $\mu$ l) were aliquot into the wells of a 96-well plate to which 100  $\mu$ l of 20% Folin-Ciocalteu reagent and 80  $\mu$ l of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution were added. The final mixture was shaken and incubated for 60 min in the dark at room temperature, and the absorbance was measured at 760 nm. The total phenolic content was calculated from the linear regression curve of Gallic acid, and the results are expressed as mg Gallic acid equivalent (GAE) per g of crude extract.

### Total flavonoid content

The total flavonoid content of the extracts was determined using the 96-well plate method, adapted from Yadav and Agarwala [22]. The extracts (100  $\mu$ l) were dispensed into the wells of a 96-well plate, and 100  $\mu$ l of 2% aluminum chloride was added. The final mixture was shaken, incubated for 15 min, and the absorbance was read at 430 nm in a microplate reader. A yellow color indicated the presence of flavonoids. The total flavonoid content was calculated from the linear regression curve of quercetin, and results are expressed as mg quercetin equivalent (QE) per g of crude extract.

## Antioxidant Activity

### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical-scavenging activity of the extracts was determined in a 96-well plate, using the modified methods of Brand-Williams *et al.* [23] and Fukumoto and Mazza [24]. To the extracts (50  $\mu$ l) in eight serial dilutions and the positive control in serial dilution (8-250  $\mu$ g/ml), 200  $\mu$ l of DPPH (25  $\mu$ g/ml) was added. The DPPH solution was prepared daily and stored in an amber bottle. The change in absorbance at 517 nm was measured after 30 min incubation in the dark, with a microplate reader. Ascorbic acid and Trolox were used as positive controls, and extract without DPPH as blank. The results are expressed as percentage reduction of the initial DPPH absorption. The concentration, of extract leading to 50% reduction of DPPH (IC<sub>50</sub>), was determined.

## Anti-inflammatory Activity

### Inhibition of 5-lipoxygenase assay

The anti-inflammatory activity of the extracts was determined by the method of Baylac and Racine [25]. Briefly, extracts (20  $\mu$ l) or positive control in serial dilutions were added to the wells of a 96-well plate. To this, 50  $\mu$ l of the 5-lipoxygenase enzyme (400 units/ml cold 2 M borate buffer, pH 6.9) was added and incubated at room temperature for 5 min. Thereafter, 50  $\mu$ l substrate solutions (10  $\mu$ l linoleic acid in 2 M borate buffer, pH 6.9) and distilled water or quercetin were added to start the reaction and incubated for another 5 min at room temperature. After this, the absorbance was taken at 234 nm. The percentage enzyme

inhibition of extract compared with the control sample was calculated using the equation given below. The results are expressed as IC<sub>50</sub>, that is, the concentration of the extracts and controls that resulted in 50% 5-lipoxygenase inhibition.

$$\% \text{Inhibition} = 100 \times \left( \frac{\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}}{\Delta A_{\text{Control}}} \right)$$

$$\Delta A_{\text{Control}} = A_{\text{Test}} - A_{\text{Blank}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test}} - A_{\text{Blank}}$$

## Glucose Utilization Activity

### C2C12 muscle cells glucose utilization

C2C12 muscle myoblast were differentiated to myotubules by the method of Drira and Sakamoto [26] and glucose utilization assay was performed by the method of Olaokun *et al.* [27]. Briefly, the previously cultured C2C12 cells in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% foetal bovine serum (FBS) and 4 mM glutamine at approximately 70% confluence were seeded (25,000 cells/ml) into the 96-well microplates. The cells were grown to confluence in the plates, and the medium was replaced with differentiating medium (DMEM supplemented with 2% horse serum and 4 mM glutamine) and incubated at 37°C in a CO<sub>2</sub> incubator. After 6 days, the medium was removed and replaced with 100 µl of DMEM supplemented with 0.25% BSA, containing plant extracts at different concentrations (31.25, 62.5, 125, 250, and 500 µg/ml). Insulin (1 µM) served as the positive control, and 0.5% DMSO served as the solvent control. After a 1 h incubation, the glucose concentration in the medium was determined by the glucose oxidase method (Sigma GAGO 20 test kit), according to instructions.

### 3T3-L1 adipocytes glucose utilization

3T3-L1 pre-adipocytes were differentiated to adipocytes by the method of Ono *et al.* [28] and glucose utilization assay was determined by the previously described method [27]. Briefly, the previously cultured 3T3-L1 pre-adipocytes in DMEM culture medium supplemented with 10% bovine calf serum (BCS) and 4 mM glutamine at approximately 70% confluence were seeded (30,000 cells/ml) into 96-well microplates. The cells were grown to confluence, and day 2 post-confluent 3T3-L1 pre-adipocytes (day 0) were incubated in differentiation medium (fresh DMEM supplemented with 10% FBS, 5 µg/ml insulin, 0.5 mmol/l 3-isobutyl-1-methylxanthine and 10 µmol/l dexamethasone). After 2 days, the medium was removed and replaced with a post-differentiation medium (fresh DMEM containing 10% FBS and 5 µg/ml insulin), and the medium was changed every other day for an additional 7 days. The medium in each of the wells was removed and replaced with 100 µl of DMEM supplemented with 0.25% BSA containing plant extracts at concentrations of 31.25, 62.5, 125, 250, and 500 µg/ml. Insulin (1 µM) served as the positive control, and the solvent control was 0.5% DMSO. All the cells were subsequently incubated for 1½ h. The glucose concentration in the medium was determined by using the glucose oxidase method (Sigma GAGO 20 test kit), according to instructions.

## Statistical Analyses

All experiments were performed in triplicate and repeated three times. The data are presented as the mean ± standard error of mean

(SEM). The statistical analyses were performed by one-way analysis of variance (ANOVA) and considered to be significantly different at  $p < 0.05$ . When the significance was found, the location of significance was determined by Bonferroni and Tukey HSD multiple comparison *post hoc* tests.

## Results

### Phytochemical screening

The total phenolic content of the acetone extracts of the three plants' species (*C. dentata*, *P. afra*, and *P. viridiflorum*) is presented in Table 2. The total phenolic content of the extract of *C. dentata* was the highest (125.12 ± 2.18 mg/g GAE) compared to the extract of *P. viridiflorum* with the lowest total phenolic content (26.08 ± 1.41 mg/g GAE). Similarly, the total flavonoid content of *C. dentata* extract was the highest (27.69 ± 4.98 mg/g QE) (Table 2), while the extract of *P. viridiflorum* exhibited the lowest total flavonoid content (20.31 ± 2.60 mg/g QE).

### Antioxidant activity

The DPPH radical scavenging antioxidant activity of the extracts was less potent than those of the positive controls ascorbic acid and trolox (Table 2). Among the three plants, the extract of *P. viridiflorum* exhibited a stronger DPPH radical scavenging ability with an IC<sub>50</sub> value of 12.94 ± 1.09 µg/ml than the other plant extracts. The *P. afra* extract exhibited the weakest DPPH radical scavenging ability with an IC<sub>50</sub> value of 32.05 ± 3.89 µg/ml. The positive controls ascorbic acid and trolox potently scavenged the DPPH radical with an IC<sub>50</sub> value of 0.21 ± 1.45 and 0.47 ± 1.38 µg/ml, respectively.

### Anti-inflammatory activity

The extract of *P. viridiflorum* was a stronger inhibitor of 5-lipoxygenase activity than the other plants, but was less potent than the positive control (Table 2). The extract of *P. viridiflorum* inhibited 5-lipoxygenase activity with an IC<sub>50</sub> value of 107.26 ± 5.63 µg/ml, while the extract of *P. afra* was the weakest inhibitor (107.26 ± 5.63 µg/ml) of 5-lipoxygenase activity. The positive control quercetin inhibited 5-lipoxygenase activity with an IC<sub>50</sub> value of 9.02 ± 2.38 µg/ml.

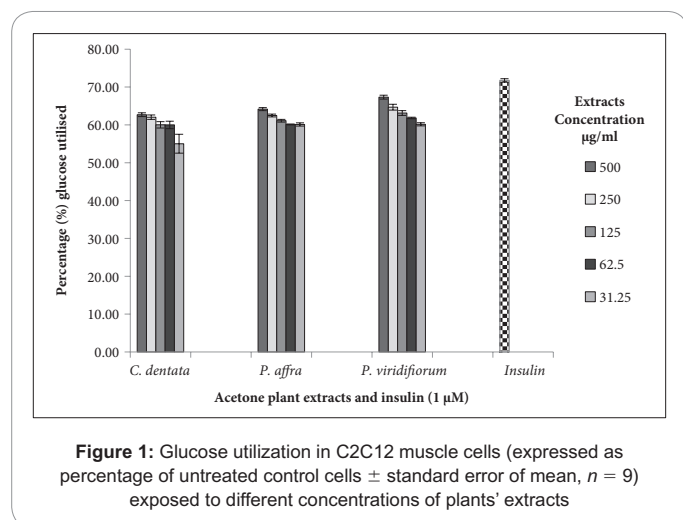
### Glucose utilization activity of C2C12 muscle cells

The effect of the acetone extracts of the three plants' species (*C. dentata*, *P. afra*, and *P. viridiflorum*) on glucose utilization of C2C12 myotubule is presented in Figure 1. All the extracts of the plants

Plant extracts	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/gQE)	Antioxidant activity (DPPH) IC <sub>50</sub> (µg/ml)	LOX inhibitory activity IC <sub>50</sub> (µg/ml)
<i>C. dentata</i>	125.12 ± 2.18	27.69 ± 4.98	22.57 ± 3.49	95.38 ± 4.04
<i>P. afra</i>	63.06 ± 1.67	22.16 ± 6.41	32.05 ± 3.89	107.26 ± 5.63
<i>P. viridiflorum</i>	26.08 ± 1.41	20.31 ± 2.60	12.94 ± 1.09	46.50 ± 4.54
Trolox	nd	nd	0.47 ± 1.38	nd
Ascorbic acid	nd	nd	0.21 ± 1.45	nd
Quercetin	nd	nd	nd	9.02 ± 2.38

Values are means of triplicate determinations performed three times ( $n = 9$ ) ± standard error; 5-LOX: 5-lipoxygenase; nd: not determined.

**Table 2:** Total phenolic content, total flavonoid content antioxidant, and 5-lipoxygenase inhibitory activity of acetone extracts of three plants' species



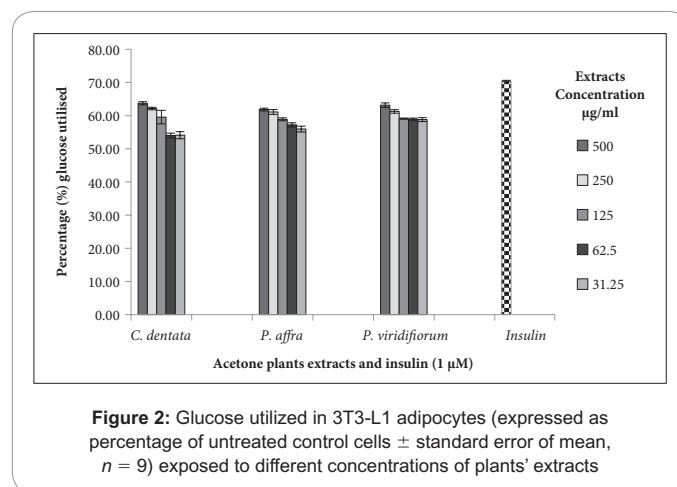
enhanced the glucose utilization activity of the C2C12 muscle cells in a dose-response manner. The extracts of *P. viridiflorum* enhanced the highest ( $67.34 \pm 0.51\%$ ) glucose utilization activity of the muscle cells at the highest concentration (500 µg/ml) with no significant difference ( $p < 0.05$ ) among the plants extracts. The glucose utilization activity of the C2C12 muscle cells by the extract of *C. dentata* and *P. affra* was  $62.69 \pm 0.50$  and  $64.17 \pm 0.40\%$ , respectively, at the highest concentration of 500 µg/ml. Insulin (positive control) at a concentration of 1 µM enhanced  $71.73 \pm 0.47\%$  glucose utilization activity of the C2C12 muscle cells.

### Glucose utilization activity of 3T3-L1 adipocytes

The effect of the acetone extracts of the three plants' species (*C. dentata*, *P. affra*, and *P. viridiflorum*) on glucose utilization of 3T3-L1 adipocytes is presented in Figure 2. All the extracts enhanced the glucose utilization activity of 3T3-L1 adipocytes with no significant difference ( $p < 0.05$ ) among them. The extracts of *C. dentata* and *P. viridiflorum*, respectively, enhanced  $63.72 \pm 0.48\%$  and  $63.15 \pm 0.68\%$  glucose utilization activity of 3T3-L1 adipocytes at the highest concentration of 500 µg/ml. Insulin, the positive control, enhanced  $70.45 \pm 0.22\%$  glucose utilization activity of 3T3-L1 adipocytes at the concentration of 1 µM.

### Discussion

Medicinal plants are potential sources of new compounds for the control or treatment of diseases which, in turn, could be the sources of lead compounds for drug development [29]. The leaves of *C. dentata*, *P. affra*, and *P. viridiflorum* were used for this study, because they are a renewable source of materials for the production of plant medicines or herbal remedies. These leaves were crushed to powder to reduce the particle size and were extracted with acetone. This differed from the traditional practice where herbal remedies would be prepared with either water or alcohol [30]. Acetone, being a solvent of intermediate polarity, has the capacity to extract a greater variety of compounds. The larger the variety of compounds that is extracted by solvents, the broader will be the expected spectrum of biological activity and the biologically active compounds that should be extracted. Unfortunately, in systems where water and alcohol are the extraction solvents, mainly polar compounds will be extracted thus limiting the amount of active compounds obtainable from plants.



Similar to the polar solvents (water and alcohol), acetone can extract polyphenols but with fewer compounds (sugars) that can interfere with bioassays, which measure glucose concentration [32,33]. Polyphenols are phytochemicals that are recognized for their potential health benefit. Studies indicate that the consumption of food and beverages rich in polyphenols are associated with significant reduced risks for a variety of non-communicable diseases and are considered to be an important component of the human diet [34]. The total polyphenol content of *C. dentata* ( $125.12 \pm 2.18$  mg/g GAE) acetone extract was the highest among the three plants investigated. The polyphenols are a large and heterogeneous group of phytochemicals in plants that comprise the groups of flavonoids and phenolic acids. The flavonoid group of compounds shares a common structure that consists of two aromatic rings (A and B), bound together by three carbon atoms that form an oxygenated heterocycle (ring C) [35]. They are widely distributed in plants and have been demonstrated to exhibit a variety of biological activities, including antioxidant, anti-inflammatory, and antidiabetic. The total flavonoid content of the selected plants' species evaluated as quercetin equivalent, resulted in *C. dentata* containing the highest ( $27.69 \pm 4.98$  mg/g QE) among the plants investigated. There is limited information on the quantitative phytochemical content of *C. dentata*. One study reported that *C. dentata*'s hydroalcoholic extract contains a total polyphenol content of  $14.86 \pm 0.05$  mg/g tannic acid equivalent and total flavonoid content of  $13.64 \pm 0.03$  mg/g QE [17]. The lower total polyphenol and flavonoid content may be due, in part, to the solvent of extract [31].

Considerable evidence abound indicate that increased oxidative damage is associated with/and may contribute to the development of degenerative diseases and age-related diseases including diabetes [36]. Plant antioxidants may limit this damage by acting directly on reactive oxygen species or by stimulating endogenous defense system [37]. Polyphenols have been attributed to possess disease protective effects owing to their antioxidant ability [36]. Coupled with this, the phenolic groups in the polyphenols can accept electrons to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components [37]. The antioxidant potency of *C. dentata*, *P. affra*, and *P. viridiflorum*'s extracts was evaluated by the ability to scavenge DPPH radicals, and the extract of *P. viridiflorum* ( $12.94 \pm 1.09$  µg/ml) was the most potent scavenger. There was no correlation between the phytochemical content and antioxidant activity of the extracts. The conflicting results have been reported by other authors. While

some authors found correlation between the polyphenol content and antioxidant activity, others found no relationship. However, studies have shown that high polyphenol content does not always translate to high antioxidant activity [38]. Moreover, the antioxidant efficiency of the polyphenol depends, in part, on the position and extent of hydroxylation and conjugation [39].

Polyphenolic extracts with high antioxidant activity are speculated to exhibit potent anti-inflammatory activity [40]. The reason may be attributed to ROS being an underlining factor inducing both oxidative stress and inflammatory cytokines [41]. The anti-inflammatory activity of the acetone extracts of the plants was evaluated by the inhibition of 5-lipoxygenase activity. These are lipid peroxidizing enzymes involved in the biosynthesis of inflammatory lipid mediators such as leukotrienes and prostaglandins. They catalyze the addition of molecular oxygen into poly-unsaturated fatty acids such as arachidonic and linoleic acids [42]. In this study, no correlation was found between phytochemical content and anti-inflammatory activity of the extracts. The potent anti-inflammatory ability by inhibition of 5-lipoxygenase activity is enhanced when polyphenols possess an 11-keto group and a hydrophilic function in the pentacyclic ring system [43]. Despite the extract of *P. viridiflorum* having the lowest total polyphenol content, the extract exhibited the strongest anti-inflammatory activity among the plants' extracts evaluated. In addition, there seems to be a relationship between antioxidant and anti-inflammatory activity of the extract of *P. viridiflorum*. The implication of this is that polyphenolic compounds alone may likely not be responsible for the superior antioxidant and anti-inflammatory activity of the extract of *P. viridiflorum*. In addition, according to the report of Kamatou *et al.* [43], the extracts of these plants may possess moderate anti-inflammatory activity. However, it is important to note that the moderate anti-inflammatory activity of these plants does not imply that the extracts may not possess stronger anti-inflammatory activity, if evaluated in other anti-inflammatory bioassays.

With glucose being the main energy fuel requirement of the body, it has to be transported from the blood across the cell membrane into the cellular cytoplasm for it to be utilized by the cells. However, the membrane of cells is impermeable to glucose, and thus the cell is reliant on trans-membrane proteins transporters (GLUT) for diffusion into the cell [44]. The impairment in the regulation of blood glucose level may lead to an imbalance that may lead to hyperglycemia thus Type 2 diabetes necessitating proper management [41]. Polyphenols are plant chemicals that possess hypoglycemic potentials [45]. One mechanism through which this group of chemical modulate glucose metabolism is to stimulate the muscle and fat cells to increase their glucose utilization activities. We investigated the effect of *C. dentata*, *P. afra*, and *P. viridiflorum* extracts on the glucose utilization activities of C2C12 muscle cells and 3T3-L1 adipocytes. The result revealed that the acetone extracts of the selected plants in dose responsive manner enhanced the glucose utilization activities of the muscle and fat cells to the same extent. This indicates that the extracts of the plants' species possessed the potential to overcome insulin resistance in the cells. Insulin resistance is present when the biological effect of insulin is not sufficiently potent to enhance the glucose uptake of the muscle cells and adipocytes [46]. No relationship was found between the phytochemical content and the glucose utilization activity of the plant extracts. However, to some extent, for the extract of *C. dentata*, there is a possible relationship between the phytochemical content and the glucose utilization activity of the 3T3-L1 adipocytes. Some studies reported that, for some plant extracts at least, a positive correlation exists between antidiabetic

activity and flavonoid content [47,48]. There is also a possibility that the compounds facilitating the glucose utilization activity of the C2C12 muscle cells may be different from that stimulating the glucose utilization activity of the 3T3-L1 adipocytes. Although the mechanism by which the selected plants' extracts enhanced glucose utilization activities of the cells was not investigated, the limiting step of insulin stimulated the glucose uptake of muscle and fat is the translocation of GLUT4 transporters to the plasma membrane [49]. Studies showed that polyphenols are able to enhance the glucose utilization activity by either acting as insulin mimetic [50,51] or without affecting the insulin receptor autophosphorylation and/or transporter translocation to the plasma membrane [45]. One of these mechanisms might have been the action mode by which the plants' extracts enhanced the glucose utilization activities of the cells. This study was the first to demonstrate that the extracts *C. dentata*, *P. afra*, and *P. viridiflorum* have the potential to enhance the glucose utilization activities, thus attenuating hyperglycemia. As the extract of *C. dentata* is used traditionally to treat obesity, the result of this study further corroborated that it is used in the treatment of obesity-related disorders such as diabetes. Obesity is one of the factors provoking insulin resistance in the responsive cells. With the polyphenolic compounds of *C. dentata* enhancing superior glucose utilization activity of 3T3-L1 adipocyte, the plant would have been one potential source of therapeutic agent for diabetes. Unfortunately, no further investigation can be conducted as this plant is a threatened species [52]. Similarly, the extract of *P. viridiflorum* is used in traditional medicine to treat inflammatory conditions. Inflammation, especially activated by obesity, is demonstrated to be one of the causes of insulin resistance in a diabetic state. The extract of *P. viridiflorum* contained phytochemicals possessing anti-inflammatory activity and enhanced the superior glucose utilization activity of C2C12 muscle cells. Therefore, the extract of *P. viridiflorum* can be further investigated as a potential source for hypoglycemia remedies and compounds.

## Conclusion

*C. dentata*, *P. afra*, and *P. viridiflorum* are medicinal plants used traditionally in South Africa to treat diseases. This study investigated the effects of the polyphenols and flavonoids of acetone extracts of these plants' species on glucose utilization activity of muscle and fat cells, anti-inflammatory, and antioxidant activity. All the extracts of the plants contained various amounts of polyphenols and flavonoids. In general, there is no relationship between the phytochemical contents of the plants' species and the antioxidant, anti-inflammatory, and glucose utilization activities. For the extract of *C. dentata*, the polyphenols and particularly flavonoids may likely be responsible for the superior glucose utilization activity of 3T3-L1 adipocytes. For the extract of *P. viridiflorum*, other compounds that are not polyphenols are likely to be responsible for the superior anti-inflammatory and antioxidant activities as well as the glucose utilization activity of C2C12 muscle cells. This study demonstrated the possibility of plants used frequently to treat diseases as potential therapeutic sources for diabetes.

## Competing Interests

The authors declare that they have no competing interests.

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