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*Full Length Research Paper*

# Screening of African traditional vegetables for their alpha-amylase inhibitory effect

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**Diabetes is a syndrome of disordered metabolism resulting in abnormally high blood sugar levels (hyperglycemia). The one possibility of lowering postprandial glucose levels is by the inhibition of α-amylase activity. In this study, aqueous extracts from leaves, fruits and flowers, stems and roots of twenty African traditional vegetable plants were tested for their inhibitory effect on α-amylase. The results showed that leaves of *Centella asiatica* (3 and 5 mg/ml) and *Ceratotheca triloba* (5 mg/ml), roots of *Cleome monophylla* (5 mg/ml), fruits and flowers of *Amaranthus hybridus* (3 mg/ml), *Justicia flava* (3 mg/ml) and *Chenopodium album* (3 mg/ml), stem parts of *J. flava* (3 mg/ml), *Portulaca oleracea* (3 mg/ml) and *C. monophylla* (3 mg/ml) showed significant (more than 70%) reduction in α-amylase activity. Though results from this study showed significant α-amylase inhibition at higher concentrations (3 and 5 mg/ml), traditionally used anti-diabetic medicinal plants are found to have α-amylase inhibition at very low concentrations, mostly less than 1 mg/ml. These traditional plant species regularly consumed as vegetables by rural people, will not only give dietary nutritional benefits, but also play vital role in inhibiting α-amylase activity, thereby reducing the blood glucose level and benefiting diabetic patients.**

**Key words:** African leafy vegetables, anti-diabetic, α-amylase, inhibitory effects.

## INTRODUCTION

Many developing countries around the world use traditional medicine, in particular herbal medicine, because it is sometimes the only affordable source for healthcare (Bhattarai, 1993; Manandhar, 1995). As for the developed countries, the use of herbal medicine for chronic diseases is encouraged because there is concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer more gentle means of managing such diseases (WHO, 2002). Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and are relatively low in cost.

Diabetes mellitus is the most common endocrine disease worldwide and about 173 million people suffer world wide. Diabetes causes about 5% of all deaths globally each year and 80% of the people with diabetes live in low and middle income countries. Diabetes deaths are likely to increase by more than 50% in the next 10 years unless urgent action is taken (WHO, 2008). Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM / Type II). Many and diverse therapeutic strategies for the treatment of Type II diabetes are known. Conventional treatments include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides (Groop et al., 1997, Perfetti et al., 1998). The α-glucosidase enzymes such as α-amylase are responsible for the breakdown of oligo and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood

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glucose level, because the monosaccharides are the form of carbohydrates which are absorbed through the mucosal border in the small intestine. The known glucosidase inhibitors which are conventionally used in the management of diabetes are acarbose and miglitol, however, these drugs are known to be associated with gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients (Fujisawa et al., 2005; Singh et al., 2007). Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having fewer side effects.

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports about 800 plants that may possess anti-diabetic potential (Alarcon-Aguilara et al., 1998). A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM (Bailey and Day, 1989; Ivorra et al., 1988; Marles and Farnsworth, 1995). There are more than 200 pure compounds from plant sources that have been reported to show blood glucose lowering activity (Marles and Farnsworth, 1994).

The traditional African herbal medicinal system practiced for over thousands of years have reports of anti- diabetic plants with no known side effects. Such plants and their products have been widely prescribed for diabetic treatment all around the world with less known mechanistic basis of their functioning. Hence, these natural products need to be evaluated scientifically in order to check for their anti-diabetic properties. In this study, the anti-diabetic effects of twenty local African vegetable plants were investigated for their ability to inhibit α-amylase activity.

## MATERIALS AND METHODS

### Plant materials and sample collection

Twenty locally consumed leafy vegetables used in this study were collected from the Durban area in Kwa-Zulu Natal, South Africa. The details of these species have been tabulated in Odhav et al. (2007). The plant species were brought to the laboratory in refuse bags and were processed.

### Preparation of plant extracts

Upon receipt, the leaves, roots, fruits and flowers were separated and washed several times with distilled water until no foreign material remained and air dried for 24 h. Thereafter, they were oven dried in an oven (Memmert, South Africa) at 25°C for 7 days. The plant parts were powdered using an industrial grinder (Retsch Gmbh, West Germany) and the powder stored in Schott bottles until use.

The aqueous extraction of the dried plant material was carried out according to Jeremy and Whiteman (2003) with modifications. Fifty grams of the dried plant material was stirred in 200 ml of distilled water. This was placed in a rotary shaker for 24 h before centrifuging (Centrifuge 5810R V5.8) at 8000 rpm for 10 min. The resultant supernatant was filtered using Whatman No. 1 filter paper. The crude extract was subsequently oven dried at a temperature of 35°C to form a powdery residue. The powdery dried crude extract was dissolved in solvents for further analyses.

### Assay for α-amylase inhibition

The α-amylase inhibition was assayed by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as the decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method of Bernfeld (1955) from Sigma-Aldrich (www.sigmaaldrich.com) was followed to estimate the maltose equivalent. One ml of plant extract was pre-incubated with α-amylase -1 U/ml (Sigma) for 30 min and 1 ml (1% w/v) starch solution was added. This was further incubated at 37°C for 10 min. The reaction was stopped by adding 1 ml DNS reagent (12.0 g of sodium potassium tartrate, tetrahydrate in 8 ml of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and then the contents were heated in a boiling water bath for 5 min. A blank was set without plant extract and another without amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium Phosphate buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20°C). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. Acarbose (Bayer Healthcare) was used as positive control.

Preliminary experiments were conducted to establish optimal assay conditions (temperature, substrate enzyme and inhibitor concentration). The aqueous plant extracts from different plant parts were diluted in buffer to give a final concentration of 3000 µg/ml, 2000 µg/ml and 1000 µg/ml. The anti-diabetic activity was investigated through the inhibition of α-amylase, an enzyme that assisted the digestion of starch and so reduced the glucose absorption. The α-amylase inhibition was expressed as a percentage of inhibition and calculated by the following equations:

$$\% \text{ reaction} = (\text{maltose})\ \text{test} / (\text{maltose})\ \text{control} \times 100$$

$$\% \text{ inhibition} = 100 - \% \text{ reaction}$$

### Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

## RESULTS AND DISCUSSION

The aqueous extracts were prepared from leaves, roots, fruits and flowers and stems from twenty local African vegetables. The results demonstrate that several plants are able to inhibit α-amylase activity. Ancient literatures, like Ayurveda reported herbal extracts being used as anti-diabetics which are directly or indirectly used for the preparation of many modern drugs. However, these plants have not gained much importance as medicines and one of the factors is lack of specific standards being prescribed for herbal medicines and supportive animal/ clinical trials (Gupta, 1994). Plants are known to produce a large variety of glucosidase inhibitors that provide protection against insects and microbial pathogens (Ryan, 1989; Lu et al., 1999). In the present study,

**Table 1.** α- Amylase inhibitory activity of leaf extracts of different plant species.

| Scientific name | % inhibition | | |
|---|---|---|---|
| | 1 mg/ml | 3 mg/ml | 5 mg/ml |
| **Leaves** | | | |
| *A. dubius* | 22.31 ± 0.10 | 43.21 ± 0.12 | 74.58 ± 0.22 |
| *A. hybridus* | 22.85 ± 0.14 | 52.76 ± 0.17 | 82.57 ± 0.21 |
| *A. spinosus* | 6.47 ± 0.11 | 11.32 ± 0.09 | 49.52 ± 0.16 |
| *A. gangetica* | 5.27 ± 0.07 | 25.75 ± 0.11 | 66.53 ± 0.32 |
| *B. pilosa* | 13.96 ± 0.17 | 48.91 ± 0.21 | 92.75 ± 0.34 |
| *C. asiatica* | 20.19 ± 0.32 | 96.22 ± 0.29 | 98.87 ± 0.37 |
| *C. triloba* | 43.23 ± 0.29 | 99.31 ± 0.52 | 99.07 ± 0.38 |
| *C. album* | 4.29 ± 0.11 | 5.76 ± 0.13 | 32.52 ± 0.24 |
| *C. monophylla* | 13.41 ± 0.41 | 35.73 ± 0.32 | 68.93 ± 0.29 |
| *Emex australis* | 6.91 ± 0.18 | 22.16 ± 0.07 | 54.69 ± 0.21 |
| *G. parviflora* | 16.80 ± 0.11 | 41.22 ± 0.18 | 93.69 ± 0.09 |
| *J. flava* | 14.65 ± 0.24 | 13.53 ± 0.32 | 51.66 ± 0.19 |
| *Momordica balsamina* | 8.85 ± 0.07 | 21.34 ± 0.01 | 55.38 ± 0.24 |
| *O. sinuatum* | 13.05 ± 0.33 | 70.26 ± 0.52 | 90.52 ± 0.47 |
| *Physalis viscosa* | 15.90 ± 0.22 | 35.24 ± 0.17 | 75.08 ± 0.26 |
| *P. oleracea* | NI | 7.41 ± 0.16 | 75.37 ± 0.30 |
| *S. occidentalis* | 5.76 ± 0.09 | 20.94 ± 0.21 | 81.94 ± 0.42 |
| *S. nodiflorum* | 16.52 ± 0.20 | 44.21 ± 0.18 | 87.33 ± 0.08 |
| *S. oleraceus* | 6.51 ± 0.38 | 10.44 ± 0.14 | 73.75 ± 0.36 |
| *T. officinale* | 25.03 ± 0.29 | 42.77 ± 0.30 | 71.95 ± 0.24 |
| Acarbose (1 mg/ml) | | 99.21 ± 0.32 | |

different plant organs of twenty African plants traditionally used as leafy vegetables were investigated for their potential to inhibit α-amylase activity. As such plant extracts were analyzed for α-amylase inhibitory activity.

Three different concentrations *viz.,* 1, 3 and 5 mg/ml of leaf extracts were tested for the inhibition of α-amylase activity (Table 1). At 1 mg/ml concentration, *Ceratotheca triloba* had the highest α-amylase inhibition of 43.23% followed by *Taraxacum officinale, Amaranthus hybridus* and *Amaranthus dubius* with the inhibition of 25.03, 22.85 and 22.31%, respectively. The concentration of 3 mg/ml of *C. triloba* extract showed the highest inhibition of 99.31%, followed by *Centella asiatica* and *Oxygonum sinuatum* which showed the inhibition of 96.22 and 70.26%, respectively. The inhibition from the rest of the samples was below 60%. The aqueous extracts of *C. triloba* at 5 mg/ml concentration again led to the highest inhibition of 99.07% compared to the rest of the other leaf samples. *C. asiatica, Galinsoga parviflora, Bidens pilosa* and *O. sinuatum* which showed inhibitory activity of 98.87, 93.69, 92.75 and 90.52%, respectively at 5 mg/ml concentration. Root samples from four plant species were tested for their inhibitory effect on α-amylase activity (Table 2). The root sample of *Cleome monophylla* at 1 mg/ml concentration showed 20.70% enzyme inhibition, which is the highest inhibitory activity observed. In 3 mg/ml concentration, *Sonchus oleracea* led to 82.31% enzyme inhibition and the rest of the other root samples led to the inhibitory activity of below 60%. There was a high inhibitory activity in 5 mg/ml with *Solanum nodiflorum* showing 99.07% followed by *C. monophylla, S. oleracea* and *G. parviflora* with percentage inhibition of 98.03, 72.57 and 62.76 respectively.

The aqueous extract from fruit and flower parts of eight plant species (Table 2) revealed that the highest inhibition at 1 mg/ml was observed in *A. hybridus* (34.22%). *Chenopodium album* at 3 mg/ml concentration showed the highest inhibitory activity of 98.72%, followed by *Justicia flava, A. hybridus* and *C. monophylla* with the percent inhibition of 96.45, 92.50 and 80.03, respectively. The highest inhibition of α-amylase activity was observed with *S. nodiflorum* at 5 mg/ml concentration, where the percent inhibition was 91.61. *A. hybridus, J. flava* and *B. pilosa* had an inhibitory effect of 87.55, 85.75 and 80.95%, respectively.

The α-amylase inhibitory effects of ten stem aqueous extracts and one seed aqueous extract were tested (Table 2). For the stem extracts, the highest inhibition at 1 and 3 mg/ml was observed in *J. flava* with 45.21 and 96.81%, respectively. No inhibition was observed in *B. pilosa*, *G. parviflora* and *C. album* with 1mg/ml concentration. *Portulaca oleracea* and *C. monophylla* showed inhibition of 80.25 and 79.75%, respectively at 3 mg/ml concentration. A high enzyme inhibition of 99.60% was

**Table 2.** α- Amylase inhibitory activity of aqueous extracts from roots, fruits and flowers, stems and seeds of different plant species.

| Scientific name | % inhibition | | |
|---|---|---|---|
| | 1 mg/ml | 3 mg/ml | 5 mg/ml |
| **Roots** | | | |
| *C. monophylla* | 20.70 ± 0.19 | 40.81 ± 0.22 | 98.03 ± 0.20 |
| *G. parviflora* | 15.78 ± 0.18 | 25.66 ± 0.12 | 62.76 ± 0.09 |
| *S. nodiflorum* | 5.80 ± 0.22 | 59.61 ± 0.26 | 99.07 ± 0.28 |
| *S. oleraceus* | 1.93 ± 0.14 | 82.31 ± 0.11 | 72.57 ± 0.21 |
| **Fruits and flowers** | | | |
| *A. dubius* | NI | NI | 22.10 ± 0.30 |
| *A. hybridus* | 34.22 ± 0.21 | 92.50 ± 0.33 | 87.55 ± 0.28 |
| *B. pilosa* | 15.03 ± 0.14 | 58.61 ± 0.25 | 80.95 ± 0.44 |
| *C. album* | 3.82 ± 0.23 | 98.72 ± 0.28 | 54.52 ± 0.38 |
| *C. monophylla* | 8.76 ± 0.26 | 80.03 ± 0.46 | 51.14 ± 0.52 |
| *G. parviflora* | 6.21 ± 0.12 | 79.30 ± 0.31 | 58.03 ± 0.25 |
| *J. flava* | 7.04 ± 0.20 | 96.45 ± 0.46 | 85.72 ± 0.19 |
| *S. nodiflorum* | 15.52 ± 0.11 | 47.08 ± 0.17 | 91.61 ± 0.52 |
| **Stems** | | | |
| *A. dubius* | 4.62 ± 0.11 | 30.33 ± 0.10 | 64.33 ± 0.47 |
| *B. pilosa* | NI | 0.11 ± 0.08 | 69.60 ± 0.52 |
| *C. asiatica* | 24.21 ± 0.23 | 65.08 ± 0.44 | 87.43 ± 0.38 |
| *C. album* | NI | NI | 23.57 ± 0.22 |
| *C. monophylla* | 33.26 ± 0.21 | 79.75 ± 0.30 | 34.43 ± 0.18 |
| *G. parviflora* | NI | 17.08 ± 0.28 | 98.47 ± 0.11 |
| *J. flava* | 45.21 ± 0.16 | 96.81 ± 0.24 | 14.86 ± 0.24 |
| *P. oleracea* | 32.11 ± 0.19 | 80.25 ± 0.36 | 70.21 ± 0.32 |
| *S. nodiflorum* | 9.36 ± 0.34 | 68.52 ± 0.17 | 87.22 ± 0.20 |
| *T. officinale* | 12.50 ± 0.11 | 47.25 ± 0.29 | 93.54 ± 0.12 |
| **Seeds** | | | |
| *S. occidentalis* | 27.56 ± 0.09 | 69.06 ± 0.16 | 98.22 ± 0.24 |
| Acarbose (1mg/ml) | | 99.21 ± 0.32 | |

Data are mean ± SD of triplicates, NI- No enzyme inhibition.

observed in the concentration of 5 mg/ml stem samples of *B. pilosa*, followed by *G. parviflora* (98.47%), *T. officinale* (93.54%) and *S. nodiflorum* (87.22%). At a concentration of 5 mg/ml, seed samples of *Senna occidentalis* had an inhibition of 98.22%.

The findings revealed that stem samples of *B. pilosa*, leaf samples of *C. triloba*, seeds of *S. occidentalis* (5 mg/ml), stem samples of *G. parviflora* (5 mg/ml), root samples of *C. monophylla* (5 mg/ml), leave samples of *C. asiatica* (3 and 5 mg/ml), fruit and flower samples of *C. album* (3 mg/ml), stem, fruit and flower samples of *J. flava* (3 mg/ml) , stems sample of *T. officinale (*5 mg/ml), fruit and flower samples of *A. hybridus* (3 mg/ml), fruit and flower samples of *S. nodiflorum* (5 mg/ml), and leaf samples of *G. parviflora* (5 mg/ml) had an enzyme inhibition of over 90.0%. These results showed that a reliable, cost saving therapy using traditional plants could be a possibility to lower the problems of untreated diabetes because of lack of synthetic drugs. Among the plants studied, *T. officinale* was earlier reported as an anti-diabetic plant used in Quebec (Haddad et al., 2001). This was confirmed from our results where *T. officinale* showed more than 90% enzyme inhibition. Earlier results by Conforti et al. (2005) on extracts from the seeds of *Amaranthus caudatus* reported 50% α-amylase inhibition at a concentration of 25 µg/ml. In our study, all three species of *Amaranthus* (*A. dubius*, *A. hybridus* and *A. spinosus*) showed very low inhibition (≤ 22%) even at a concentration of 1 mg/ml. This may be due to the use of leaf extract in our study compared to seeds used in the earlier reports. Because of significant α-amylase inhibition, these vegetables consumed regularly by rural

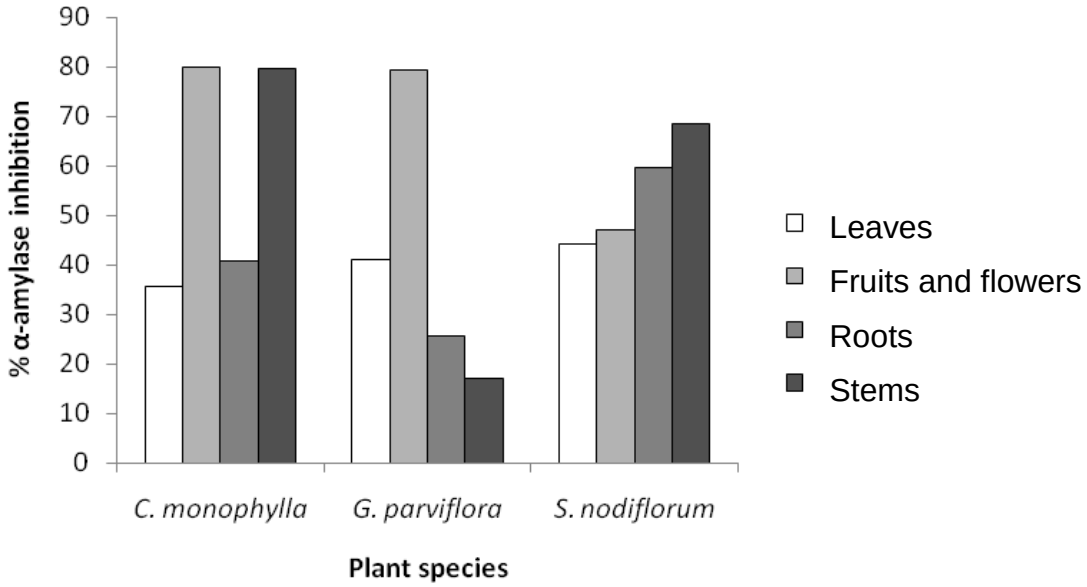


**Figure 1.** Comparison of α-amylase inhibitory effect of different plant parts from three different plant species.

people in places where insulin is not available, these plants may offer some control against diabetes.

## Conclusion

Twenty leaf samples, four root samples, eight fruit and flower samples and ten stem samples from different traditional vegetables were analyzed for their inhibitory effect on α-amylase. The results showed that leaves from *C. asiatica* and *C. triloba*, roots from *S. oleraceus*, fruits and flowers from *A. hybridus*, *J. flava* and *C. album*, stem parts from *J. flava*, *P. oleracea* and *C. monophylla* showed significant inhibition. In most of the earlier reports, the traditionally known anti-diabetic plants have been studied but in our study traditional leafy vegetables were investigated for their α-amylase inhibitory activity. Results of this study showed significant α-amylase inhibition at very high concentrations of plant extracts. In the quest for anti-diabetic compound from plants, the focus has been on medicinal plants used traditionally and these studies reported the inhibition of α-amylase at very low concentrations (less than 1 mg). Although this study reports α-amylase inhibition at higher concentration, these leafy vegetables offer a safe method to control or supplement treatment strategy through its α-amylase inhibitory effect in addition to beneficial nutritional effects (Figure 1).

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