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Full Length Research Paper

Activities of some enzymes, enzyme inhibitors and antinutritional factors from the seeds of sponge gourd (*Luffa aegyptiaca* M.)

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Activities of some of the enzymes; enzyme inhibitors and antinutritional factors of the seeds of *Luffa aegyptiaca* were investigated. Enzymes were extracted and their activities determined using standard procedures. The activities of trypsin and amylase inhibitors were also determined using standard procedures. While antinutritional factors were detemined using the AOAC method as well as standard procedures. The activies of β-amylase and phytase were low. High activities were observed in peroxidase and urease. While lipoxygenase exhbited the highest activity. Trypsin inhibitor had a higher activity than amylase inhibitor. The concentrations of phytic acid, lectins and tannnin levels were much higher compared to that of saponins. The highest concentration was observed in sapogenin. The observed amylase inhibitor activity may be responsible for the use of the seeds in the management of diabetes mellitus, however further work is needed in this area. Further work is also recommended on the separation of the sapogenin into its different components.

Key words: Lipoxygenase, β-amylase, trypsin, sapogenin.

INTRODUCTION

Luffa aegyptiaca M. belongs to the family of cucurbitaceae. Its origin can be traced to tropical Asia (Siqueira et al., 2010). It is a crawling plant that grows in the wild and on abandoned building structures and fence walls in towns and villages in Nigeria (Dairo, 2008). It is a climbing annual wild vine with lobed cucumber-like leaves that are dark green in colouration with rough surface. The plants with yellow flowers bear fruits that are cucumber shaped but larger in size and contain fibrous sponge in which the hard black seeds are enmeshed (Dairo, 2008). It is a lignocellulosic material composed of 60% cellulose, 30% hemicellulose, and 10% lignin (Mazali and Alves, 2005). It has been discovered that the consumption of sponge gourds can supply some antioxidant constituents to human body (Oboh and Aluyor, 2009). In oriental medicine, *L. aegyptiaca* has effect on the treatment of fever, enteritis and swell etc. The extracts from vines alive are used as an ingredient in cosmetics and medicine (Lee and Yoo, 2006). Immature fruit is used as vegetables, which is good for diabetes (Bal et al., 2004). One of the main uses of sponge gourd is as sponges, but they are also used as filler in the production of composites materials, materials of absorption in water treatments stations during the step of ion exchange (Tanobe et al., 2005). Its use in the cosmetic industry for the production of various bath and cosmetics products has been reported (Davis and Decourley, 1993).

Seeds are well known rich sources of minerals but the bioavailability of these minerals is usually low due to the presence of antinutrients and enzyme inhibitors (Valencia et al., 1999). These antinutrients and enzyme inhibitors interfere with absorption of nutrients from foodstuff thus affecting their metabolism. Enzymes are also present in seeds which aid digestion when consumed, as well as serve as nutrition source during germination.

There exist little or no data on the enzyme activities,

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enzyme inhibitors and antinutritional factors of the seeds of sponge gourd. Therefore this present study aims at ascertaining some of the enzymes; enzyme inhibitors and antinutritional factors of the seeds of *L. aegyptiaca*, in order to provide data, either for practical use or for basic research needs. This study will contribute to the knowledge of the seeds of this vegetable that could improve its uses.

MATERIALS AND METHODS

Plant material

L. aegyptiaca was collected from the bush at Ojo community in Lagos, Nigeria. The coats (covering) were removed and the seeds grounded into smooth powder. The powdered sample was defatted in a soxhlet extractor using hexane as solvent.

Enzyme extraction and determination

The defatted flour was homogenized with distilled water at 0 to 4°C for 10 min followed by centrifugation to provide a clear supernatant as an enzyme extract. Activities of β-amylase (Swain and Dekkar, 1966), Urease (Hofman, 1963) and lipase (Marchis-Mauron et al., 1959) were determined and expressed as µmol of respective product liberated per minute.

Lipooxygenase activity was assayed at 234 nm by the spectrophotometric method of Ben-Azis et al. (1970). Unit lipooxygenase activity corresponds to a change in absorbance of 0.001 min^{-1}.

Peroxidase activity was determined spectrophotometrically at 420 nm using o-phenylenediamine and hydrogen peroxide (Gregory, 1966). A change in absorbance of 1×10^{-3} min^{-1} corresponds to unit activity.

Phytase was assayed by measuring the rate of increase in inorganic phosphorus using the ascorbic acid method (Watanabe and Olsen, 1965).

Determination of enzyme inhibitors

For trypsin inhibitor, the defatted powdered sample was extracted using dilute alkali and the activity was determined as described by Kakade et al. (1969). Amylase inhibitor activity was determined from the aqueous extracts of the defatted powdered sample against a standard enzyme preparation of known activity at pH 6.0 and 37°C and expressed as for trypsin inhibitor (Marshall and Lauda, 2006).

Determination of antinutritional factors

Phytic acid was extracted from the defatted powdered sample with tricholoroacetic acid and estimated as ferric phytate by the method of Wheeler and Farrel (1971).

Lectin activities were quantified by using the hemagglutination method described by Aregheore et al. (1998). Lectin content was expressed in terms of the dilution value, 2^n where n represents the number of dilutions performed affecting agglutination.

Tannin was estimated by the standard AOAC (1997) method. Saponin and sapogenin content of the sponge gourd seeds (dehulled) were determined by the method of Gestetner et al. (1966). Defatted soybean flour was used as a standard.

Table 1. Activities of some enzymes in the dehulled seed of *L. aegyptiaca.*

Enzyme	Dehulled seeds (Units/mg protein)
β-amylase	0.04
Urease	24.30
Lipase	0.11
Lipooxygenase	29.00
Peroxidase	14.30
Phytase	0.03

Values are mean of triplicates.

RESULTS AND DISCUSSION

This paper reports the enzyme activities of *L. aegyptiaca* seeds and its inhibitors. Living cells perform a multitude of chemical reactions very rapidly because of the participation of enzymes. Plants in their natural states contain enzyme that they made for their own use. However, changes in the activities of these enzymes have been shown to accompany seed deterioration, which precedes loss of seed viability (Sharma and Kumar, 2010).

The activities of the enzymes of *L. aegyptiaca* are shown in Table 1. β-amylase activity was observed to be quite low. This can be attributed to the dormancy of the seed. It is known that amylase activities are usually enhanced during germination (Awoyinka and Adebawo, 2008). Zhang and Wang (2002) attributed this rise in β-amylase activities to increasing expression of the enzyme proteins. Zhang and Wang (2002) further reported that the observed rise in the activity of β-amylase is largely associated with the decline in starch concentration during fruit development. Seed ageing may also be responsible for the observed low β-amylase activity as aging is commonly accompanied by loss of enzyme activity (Ganguli and Sen-Mandi, 1993).

Urease activity was quite high as depicted in Table 1. Urease acts on urea liberating ammonia (Mertzler, 2001). Urea is usually obtained from the hydrolysis of arginine to ornithine and urea, during fermentation of seeds. Urease may play a necessary role in nitrogen metabolism (Eskew et al., 1983) as green plants have been reported to recycle nitrogen via urea and the Ni^{2+}-dependent urease (Mertzler, 2001). The observed high urease activity is of advantage to the seeds of *L. aegyptiaca* as reduction in the activities of urease leads to the accumulation of urea, which causes necrotic spots on leaves. This will further have consequences on the deficiency and metabolism of ureides, amino acids, and other organic acids (Malavolta and Moraes, 2007).

The observed high level of lipoxygenase activity shown in Table 1 may be due to tissue wounding. Lipoxygenase (LOX) is one of the most widely studied enzyme in plants and animal kingdom which is found in more than 60

Table 2. Some enzyme inhibitors in the dehulled seeds of *L. aegyptiaca*.

Enzyme inhibitor	Dehulled seed (Units/mg protein)
*Trypsin inhibitor	24.00
Amylase Inhibitor	19.60

* Extract had 48.6% inhibition for × 2.5 dilution. Values are in triplicates.

Table 3. Some antinutritional factors of the dehulled seeds of *L. aegyptiaca*

Antinutritional factor	Level
Phytic acid (mg/g)	5.16
Lectins (expressed as number of agglutination)	3.00
Tannins (g/100 g seed)	3.13
*Saponins (g/100 g seed)	0.11
§Sapogenin (mg/g seed)	83.40

*The estimated saponin value of soybean was found to be 0.487%. § Soybean sapogenin was 349 mg/g. Values are in triplicates.

species (Baysal and Demirdoven, 2007). Reports from recent studies show that lipoxygenases are involved in the mobilization of storage lipids contained in oil bodies (Heldt, 2005). It catalyses the bioxygenation of poly-unsaturated fatty acids (PUFA) containing a cis,cis-1,4-pentadiene unit to form conjugated hydroperoxydienoic acids (Baysal and Demirdoven, 2007). The observed high level of lipoxygenase activity may be an indication of a fairly high acid value of oil extracted from the seed. It is also crucial to the defence strategies of the seeds as there is ample evidence that lipoxygenase is a crucial element of plants defence strategies (Baysal and Demirdoven, 2007).

The objective of investigating phytase activity was to study the enzyme of *L. aegyptiaca* that hydrolyses phytic acid with a view to utilize this enzymatic activity for reducing the phytic acid content of the seeds. The activity was quite low at 0.3 units/mg protein (Table 1). However there is need to characterize this enzyme in order to know the conditions of its optimum activity.

Lipase hydrolyses triglycerides to glycerol and fatty acid (Pahoja and Sethar, 2002). Its presence in a seed could affect the storage stability of the oil extracted. Its activity is increased during germination of oilseeds as it hydrolyzes triacylglycerol to glycerol and fatty acids which are converted to sugars to support the growth of young sprouts (Hills and Beevers, 1987). Lipase activity and peroxidases levels of the seeds of *L. aegyptiaca* were quite high compared to other oil seeds. This observation however, contradicts reports that lipase activity is absent in dormant seeds (Enujiugha et al., 2004). Oilseed lipases have great potential for commercial exploitation as industrial enzymes, especially those oilseeds that are presently considered under-utilized, among which are the *L. aegyptiaca* seeds.

The trypsin inhibitor of the seed of *L. aegyptiaca* was quite high at 24.00 TIU/mg protein (Table 2). However this level is quite low when compared to literature values of legumes as chick pea (46.5 TIU/mg protein), lentils (40.0 TIU/mg protein), pinto bean (75.5 TIU/mg protein) and tepay bean (61.7 TIU/mg protein). Trypsin inhibitors form irreversible complexes with trypsin and inhibit its activity (Liener and Kakade, 1980). Heat treatment however has been shown to reduce trypsin inhibitor activity.

The aqueous extract of *L. aegyptiaca* seeds exhibited amylase inhibition activity of about 20 unit/mg protein (Table 2). Amylase inhibition activities of plant seeds can be attributed to defence mechanism against pest such as weevils (Huesing et al., 1991). Interference with the digestion and utilization of dietary starches by naturally occurring amylase inhibitor is of great nutritional and practical concern (Puztai et al., 1995). Purified preparations of amylase inhibitor perfused into the duodenum of humans at clinically acceptable doses have been shown to inhibit in traluminal amylase activity (Layer et al., 1985). This activity has been argued to be good in the management of non insulin-dependent diabetes mellitus as ingestion of the inhibitor with dietary starch signify-cantly reduced postprandial increases in glucose and insulin levels in both normal subjects and patients with diabetes mellitus (Boivin et al., 1988). This may be responsible for the use of the seeds in the management of the diabetes mellitus. Further work in this area is therefore recommended.

Although, oilseed meals have high protein levels and favourable essential amino acid (EAA) profiles they are known to contain a variety of growth inhibiting anti-nutritional factors (NRC, 1993; Francis et al., 2001). This acts as advantages to the plant such as resistance to some insects and soil nematodes.

The nutritional importance of phytic acid lies in its ability to chelate several mineral elements especially calcium, magnesium, iron and zinc, thereby reducing availability in the intestinal track (Hertramf and Piedad-Pascual, 2000). Phytic acid was quite high in the seeds of *L. aegyptiaca* (Table 3). This level however corresponds to reported high level of phosphorus in the seeds; since phytic acid is the major storage form of phosphorus. The observed high content of phytic acid may pose a problem of inavailibilty of micronutrients when consumed.

The level of saponin was not significantly ($p > 0.05$) high compared to that observed in soybean. Saponins are toxic to cold-blooded animals and are generally known for their bitter taste, foaming in aqueous solutions, and their ability to haemolyse red blood cells (Birk and Peri, 1980). Saponin has health benefits as well. The presence of saponin may account for the reported therapeutic actions of *L. aegyptiaca* seeds.

The sapogenin level was observed to be significantly lower ($p > 0.05$) than that of the soybean (Table 3). More

work is needed in separating the sapogenin into its different components.

The *L. aegyptiaca* seed extract was observed to affect agglutination only at low dilution values as indicated by its low lectin level (Table 3).

Conclusion

Results of this study reveal the presence of enzymes with lipoxygenase having the highest activity. Sapogenin had the highest level among the antinutritional factors investigated. The observed amylase inhibitor activity may be responsible for the use of this seeds in the management of diabetes mellitus, however further work is needed in this area. Further work is also recommended on the separation of the sapogenin into its different components.

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