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Inhibitory effects of *Launaea taraxacifolia* and *Strychnos spinosa* leaves *extract* on an isolated digestive enzyme linked to type -2 -diabetes mellitus

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Abstract

Background: The most prevalent type of diabetes mellitus (Type-2), is managed using many approaches, including the lowering of postprandial hyperglycaemia. Inhibition of key enzymes involved in carbohydrate breakdown such as *α*-glucosidase and *α*-amylase has been reported as a novel strategy to delay the absorption of glucose after meals. This study sought to determine the *in vitro* inhibitory potential of *Launaea taraxacifolia* and *Strychnos spinosa* leaf extracts on *α*-glucosidase enzyme and also determine their modes of inhibiting the enzyme.

Materials and Methods: Plant extracts were prepared using soxhlet apparatus. Inhibitory effect of extracts at different concentrations and mode of inhibition were carried out using α-glucosidase enzyme isolated from the small intestine of a guinea pig. **Results:** Extracts of *Launaea taraxacifolia* (LTE) and *Strychnos spinosa* (SSE) leaves showed α-glucosidase inhibitory potential of approximately 69 % and 79 % respectively as compared to 73 % for standard drug-acarbose at a maximum concentration of 1000 µg/mL. The IC_{50} values recorded were 205.2±0.044 µg/mL, 129.4±0.094 µg/mL and 196.9±0.036 µg/mL for LTE, SSE and acarbose respectively. The Lineweaver Burk plot showed an uncompetitive mode of inhibition for both LTE and SSE as depicted by the lower K_m and V_{max} of enzyme inhibited by extracts compared to control. **Conclusion**: Extracts of *Launaea taraxacifolia* and *Strychnos spinosa* leaves showed significant inhibitory effect on an isolated intestinal α-glucosidase enzyme in an uncompetitive mode

Keywords: *Launaea taraxacifolia, Strychnos spinosa,* α-glucosidase, diabetes mellitus

1. Introduction

One of the leading metabolic and endocrine disorders affecting a substantial number of the world's population is diabetes mellitus (Ley et al., 2014). According to Wild et al. about 180 million people globally have the disorder and it's likely to increase twofold by the year 2030. (Wild et al., 2004). Among the types of diabetes mellitus reported, type 2-diabetes mellitus, is most common (Li et al., 2004). Type-2-diabetes mellitus is managed using many pharmacotherapeutic strategies, but one that is recommended by the Third Asia-Pacific Region Diabetes Treatment Guidelines as the first-line of treatment for lowering postprandial hyperglycaemia, is the use of *α*-glucosidase inhibitors (AGIs) (Yin et al., 2014). Inhibition of enzymes like *α*-glucosidase that digest carbohydrate is a beneficial strategy to impede the

rapid absorption of glucose after meals (Bösenberg and van Zyl, 2008).

Reports indicate that there are several inimical consequences like flatulence, diarrhoea, abdominal pain and bloating associated with the use of synthetic AGIs such as Acarbose (Glucobay®), miglitol (Glyset®) and voglibose (Volix®, Basen®) (Alikunhi, et al 2012; van de Laar, 2008; Campbell et al., 1996). Implicitly it is clear that management of DM with synthetic drugs that do not pose any adverse effects is still a challenge. This has led to research attention being given to natural products with enzyme inhibitory effects. Ghana is endowed with biodiversity, especially plants and no extensive effort has been made to assess the bioactivity of these plants that are used by Traditional Medicine Practitioners (TMP) in managing diabetes mellitus. Thus more research is needed in the drug discovery process, and as such necessitated this study on *Launaea taraxacifolia*, and *Strychnos spinosa* leaves, currently used by rural folks in Ghana for managing water retention disorders, conjunctivitis, proper bone fixation in infants dyslipidaemia, hypertension and diabetes mellitus (Adebisi, 2004).

Launaea taraxacifolia is a tropical plant, which is part of the Asteraceae family and is commonly known as African Lettuce. Its leaves are used traditionally for preparing meals and also as medicine in Ghana (Adinortey et al., 2012; Dickson et al., 2012). Evidence-based reports indicate that *L. taraxacifolia* leaves possess DNA protective effect and antioxidant activity (Adinortey et al., 2018), protective effects against cisplatin and gentamicin induced kidney damage (Kuatsienu et al., 2017; Adejuwon et al., 2014) antimicrobial and antiarthritic effects (Ololade et al., 2017) and hypolipidaemic properties (Koukoui et al., 2015).

Strychnos spinosa Lam. is a member of the family Loganiaceae and is commonly known as spiny monkey orange or green monkey orange tree. Though the ripe fruit is generally edible, some consider the unripe poisonous. The plant is found throughout Africa, Madagascar, India, Sri Lanka, Southeast Asia and the northern tropical part of Australia. Different parts of the plant are used traditionally for managing several disorders including diabetes mellitus, snakebite, peptic ulcer, wounds, breast milk stimulation, venereal diseases, leprosy, diarrhoea, and malaria fever (Kabine et al., 2015; Isa et al., 2014; Asase et al., 2005; Neuwinger, 1996).

Though these plants are used in the management of diabetes mellitus, there is sparse report of their effect on enzymes that digest carbohydrate, such as α-glucosidase and α-amylase that are known to play central role in increasing postprandial hyperglycaemia. The study thus aimed at evaluating the effect of extracts of *Launaea taraxacifolia* and *Strychnos spinosa* leaves on the activity of *α*-glucosidase as well as determine their mode of inhibition.

2. Materials and Methods

2.1 Chemicals and Equipment

Para-nitrophenyl-α-D-glucopyranoside (*P*NPG) (Sigma Aldrich, Germany), para-nitrophenol (*P*NP) (Sigma Aldrich; Germany), Sodium dihydrogen Phosphate, Disodium Hydrogen Phosphate and Sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). Equipment include Labomed UVD-3200 spectrophotometer, pH meter model NOV-210 (Nova Scientific Ltd., Korea), and Sartorius analytical balance. All other chemicals

used, including solvents, were of analytical grade.

2.2 Plant Material

Plant material used were leaves of *Launaea taraxacifolia* and *Strychnos spinosa*. These plants were collected from around the environs of the University in February 2017. A curator at Herbarium section of University authenticated them.

2.3 Preparation of plant extract

Freshly harvested leaves of *Launaea taraxacifolia* and *Strychnos spinosa* were washed, cut into pieces, air dried for three weeks and subsequently put in an oven for 6 hours at 30 ^{O}C. The dried crispy leaves were then ground to fine powder and stored at 4 ^{O}C. The extraction procedure was carried out using the Soxhlet apparatus. About 100 g of powdered sample of *Launaea taraxacifolia* was weighed and put in 1L of 70 % aqueous methanol whereas *Strychnos spinosa* powder was put in 1L of 70 % aqueous ethanol. The extraction was carried out for 12 hours after which suction filtration was used to obtain a filtrate of the crude extract. The filtrate was concentrated with a rotary evaporator and dried in a desiccator for 2 weeks. The percentage yield of the extract was calculated.

2.4 Methods for qualitative analysis of phytoconstituents

Phytochemical composition of *Launaea taraxacifolia* and *Strychnos spinosa* leaves extract was analysed using the method described by Trease and Evans, 2002.

2.5 Experimental animals and maintenance

All experimental protocols were carried out with reference to the guidelines on care of experimental animals as provided by the Organization for Economic Cooperation and Development (OECD). Guinea pigs were fed on feed supplied by Ghana Agro Food Company Tema, Ghana and water *ad libitum*. They were subjected to a 12 h light and dark cycle at (25 ± 2) ^{O}C. All guinea pigs were allowed to acclimatize to their new environment for at least 14 days with adequate water and food before the start of the experiment.

2.6 Isolation of α-glucosidase enzyme from guinea pig intestine

The procedure reported by Thilagam et al. 2013 was employed. Guinea pigs were fasted for about 20 hours, sacrificed by anaesthesia and part of the small intestines immediately below the duodenum and immediately above the cecum was cut and thoroughly rinsed in ice-cold saline. The intestine was homogenized in cold solution of 0.02 M sodium phosphate (pH 6.9) buffer. The ratio of small intestine (mass) to buffer volume used for homogenization was 1:2. The homogenate was then centrifuged at 12,000 g for 30 minutes at - 4 ^{O}C and the supernatant was gently harvested and stored in polypropylene eppendorf tubes as enzyme source at -20 ^{O}C.

2.6.1 Confirmatory test for α-glucosidase enzyme activity

About 50 µL of p-Nitrophenyl-α-D-glucopyranoside (*p*NPG) solution (3 mM), which is colourless was added to 50 µL of enzyme source and incubated for 5 minutes at 25 ^{O}C. About

2 mL of 0.1 M Na_2CO_3 solution was added after incubation. The reaction mixture was observed for the formation of a yellow-coloured paranitrophenol (*P*NP) that gives an indication of the activity of α-glucosidase enzyme (Dahlqvist, 1964).

2.7 α-glucosidase inhibition assay

The inhibition potential of the plants leaf extracts on *α*-glucosidase activity was investigated according to the procedure described by Pistia-Brueggeman and Hollingsworth, (2000) with slight modification, using *α*-glucosidase obtained from guinea pig small intestine. About 50 µL of *α*-glucosidase (2.873 U/mL) was preincubated with 50 µL of different concentrations of (LTE / SSE): 200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL, 1000 µg/mL), for 10 minutes. Then 50 µL of 0.3 mM p-Nitrophenyl-α-D-glucopyranoside (*p*NPG) was added as a substrate to initiate the reaction. The reaction mixture was incubated at room temperature for 20 minutes and stopped by adding 2 mL of 0.1M Na_2CO_3. Acarbose was used as positive control and prepared in same concentrations as the extracts. It was incubated with the enzyme following the same procedure as described above. The *α*-glucosidase activity was determined by measuring a yellow-coloured paranitrophenol product released from *p*NPG at 405 nm. A negative control was prepared using the same method replacing the extract with distilled water. Blanks were prepared for each concentration with the constituent but without enzyme. The inhibitory activity of extracts on *α*-glucosidase was calculated as percentage inhibition.

% Inhibition={[ABS Control-ABS extract] /ABS Control} X 100

2.8 α-glucosidase kinetics studies

2.8.1 Estimation of α–glucosidase activity

The activity of the enzyme was determined by the procedure described by Dahlqvis, (1964) with minor modifications. A total of 50 µl of varying concentrations (1.27 mM, 2.54 mM, 3.81 mM, 5.08 mM, 6.34 mM) of the substrate, p-Nitrophenyl-α-D-glucopyranoside (*P*NPG) was incubated with 50 µl of α-glucosidase enzyme solution (2.873 U/mL) for 5 minutes at 25 ^{o}C. The reactions were stopped with 2 ml of 0.1 M Na_2CO_3 solutions. In order to determine α–glucosidase enzyme activity, absorbance of yellow coloured p-nitrophenol (*P*NP) released from *p*NPG was measured at 405 nm using a spectrophotometer. Concentration of *P*NP product released per unit time was estimated using a *P*NP standard curve and reaction rate or velocities were calculated by dividing extrapolated *P*NP concentration by 5 minutes. A graph of 1/velocity was plotted against 1/[substrate] to estimate the K_m and V_{max} of the enzyme.

2.8.2 Determination of mode of inhibition of extracts on *α*-glucosidase enzyme

The mode of inhibition of *α*-glucosidase by plant extracts was determined using the method described by Kazeem et al (2013) with minor modification. Briefly, 50 µL of the (1000 µg/mL of LT and SSE), extracts were preincubated with 50 µL of *α*- glucosidase solution (2.873 U/mL) for 10 minutes at room temperature in two different set of tubes. In different tubes, 50 µL of *α*-glucosidase enzyme was preincubated with 50 µL of sodium phosphate buffer (pH 6.9). About 50 *µ*L of *p*NPG at increasing concentrations (1.27 mM, 2.54 mM, 3.81 mM, 5.08 mM, 6.34 mM) was added to both sets of reaction mixtures to start the reaction.

The mixtures were incubated for 5 minutes at room temperature, and 2 mL of Na_2CO_3 was added to stop the reaction.

Concentration of paranitrophenol (*P*NP) released per minute was estimated by measuring absorbance of a coloured solution and extrapolating from a standard curve. The rate of reaction or reaction velocities (mM/min) were computed for by dividing each extrapolated concentration by 5 minutes. A Lineweaver-Burk graph was plotted and the kinetic parameters, Km and Vmax were estimated using the Michaelis-Menten equation.

2.9 Data Analysis

All results were presented as mean ± SEM (standard error of mean) for triplicate measurements. The IC_{50} value of the test substance was determined through a nonlinear regression analysis of the dose response curve. Data were analyzed by one-way ANOVA followed by Bonferroni posthoc test using Graphpad Prism software US version 8.

3. Results

Methanolic extract of *Launaea taraxacifolia* had a percentage yield of 14 % whilst *Strychnos spinosa* recorded 26 %. The phytochemical screening of extracts from these plants indicated the presence of terpenoids, flavonoids, tannins, saponins, reducing sugars and alkaloids (Table 1).

Table 1. Qualitative screening of plant extracts

Phytochemical	LTE	SSE
Terpenoids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Reducing sugars	+	+

+ : present- : absent, LTE: *Launaea taraxacifolia* extract. SSE*: Strychnos spinosa* extract

α-glucosidase inhibitory potential of LTE and SSE were determined as presented in fig. 1. Data analysis using ANOVA showed a significant difference ($P<0.05$) in percentage

inhibitory activity amongst SSE, Acarbose and LTE in all concentrations tested. This observation was affirmed by a posthoc Bonferroni's multiple comparison test. At a maximum concentration of 1000 µg/mL, SSE recorded a highest percentage inhibition of 78.97±0.335%, followed by acarbose with a value of 73.30±0.34%, whilst LTE showed the least percentage inhibition of 68.97±0.27%. Investigation of potency of extracts at inhibiting α-glucosidase enzyme using regression analysis showed IC_{50} values of 196.9±0.036 µg/mL, 205.2±0.044 µg/mL and 129.4±0.094 µg/mL respectively for acarbose, LTE and SSE. Results indicate that SSE appears to be the most potent glucosidase inhibitor ($p<0.05$), even better than a known standard drug-acarbose.

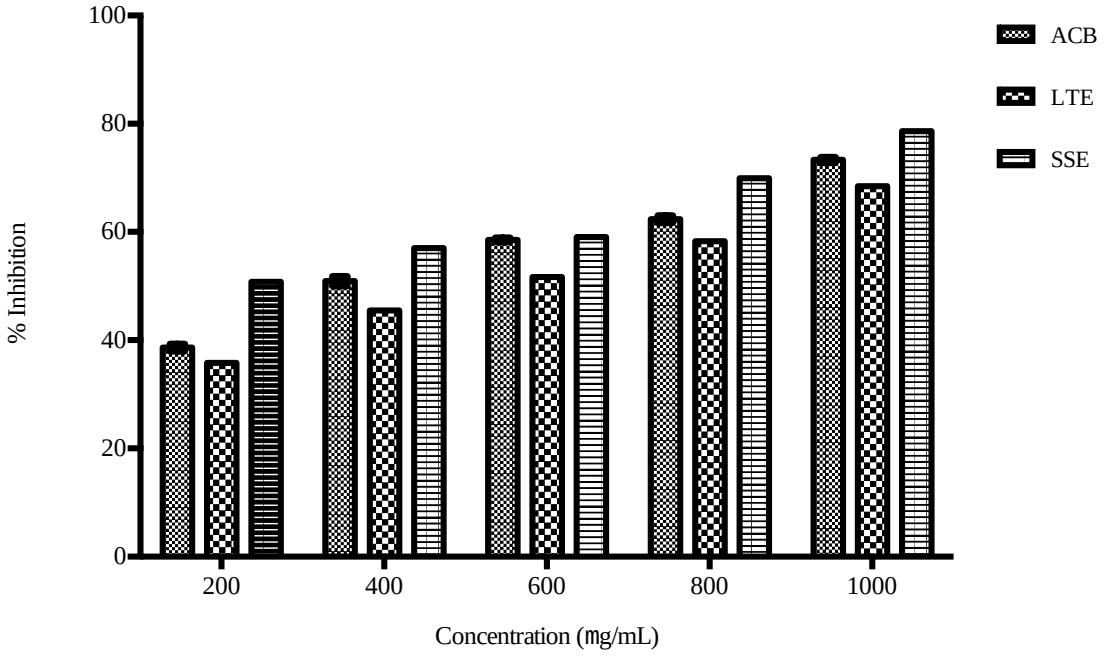

Fig. 1: Percentage inhibition of ACB, LTE and SSE on α-glucosidase enzyme.

The mode of inhibition of extracts on α-glucosidase was ascertained from the Lineweaver-Burk plot that displayed an uncompetitive mode of inhibition of the enzyme (fig. 2). The use of Lineweaver-Burk plot allowed for estimation of kinetic parameters, K_m (substrate concentration at half V_{max}) and V_{max} (maximum reaction rate) in the presence of extract and absence (control). Estimated values for K_m and V_{max} for control that is enzyme without any inhibitor-extract was 9.194 mM and 0.218 mM/min respectively. In the presence of 1000 µg/mL inhibitor, these kinetic parameters-K_m and V_{max} declined to 6.330 mM and 0.152 mM/min respectively for LTE and 1.380 mM and 0.031 mM/min respectively for SSE. The Michaelis-Menten plot in (fig. 3) indicates that, at increasing substrate (*p*NPG) concentrations, activity of α-glucosidase increases in the absence of any extract but decreases steadily when LTE or SSE of 1000 µg/mL concentration was added. This was manifested explicitly at a substrate concentration of 4 mM for both extracts (fig.3).

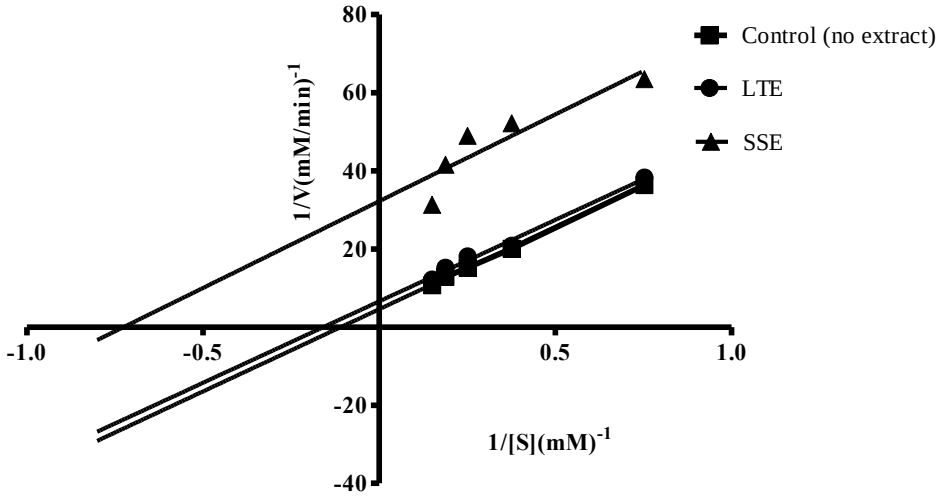

Fig 2: Lineweaver-Burk plot on the effect of LTE and SSE on α-glucosidase

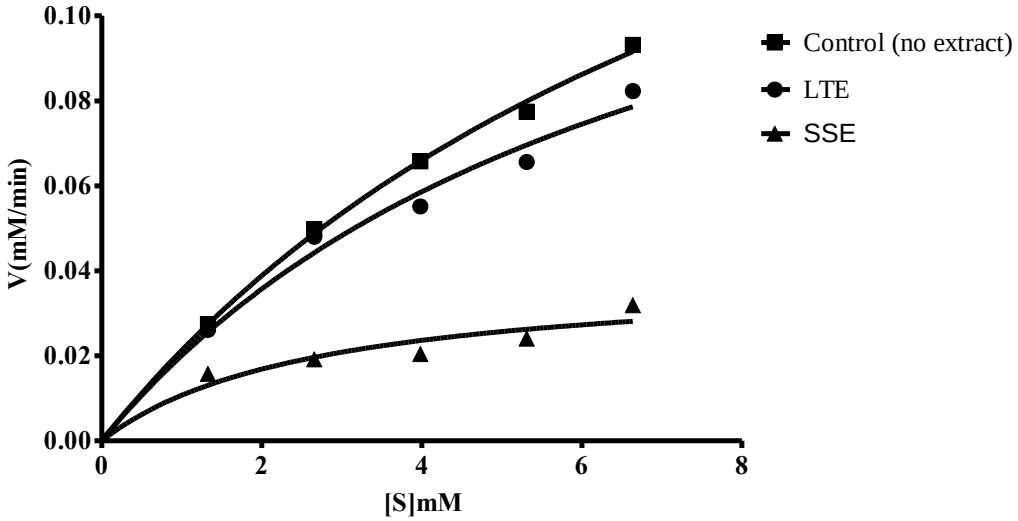

Fig 3. Michaelis-Menten curve on the effect of LTE and SSE on the activity of α-glucosidase

4. Discussion

Glucosidase is an intestinal brush border enzyme, that catalyses the release of monosaccharaides and eventually its absorption in the small intestine (Van Beers et al 1995). Glucosidase is also considered a key enzyme in carbohydrate digestion. Synthetic glucosidase inhibitors are marketed as therapeutic drugs for Type-2- diabetes mellitus. Studies have however shown that, apart from these conventional treatment methods, natural remedies from medicinal plants are considered safe and effective alternatives in managing diabetes mellitus (Medagama et al., 2014). This research therefore sought to particularly assess the inhibitory property of two locally used plants; *Launaea taraxacifolia* and *Strychnos spinosa* used to manage persistent hyperglycaemia.

Extracts of *Launaea taraxacifolia* (LTE) and *Strychnos spinosa* (SSE) leaves demonstrated a marked α-glucosidase inhibitory potential of approximately 69 % and 79 % respectively as compared to 73 % for standard drug-acarbose at a maximum concentration of 1000 µg/mL. Studies conducted elsewhere have reported that α-glucosidase inhibitory activity of acarbose ranges from 55 to 82 % (Mohamed et al., 2012; Perez-Gutierrez et al., 2012). The observation regarding acarbose percentage inhibition in this study is not contrary to what have been reported by Mohamed et al., (2012) and Perez-Gutierrez et al., (2012). SSE recorded the highest percentage inhibition at the maximum concentration used in this study. The IC_{50} value, which is the concentration of test substance at which 50 % of enzyme activity is inhibited, was significantly different amongst the three different test substances. The significant difference in these values depicts the fact that SSE could be better than LTE and acarbose during pharmacotherapy. The IC_{50} values of *launaea taraxacifolia* and *Strychnos spinosa* leaves extract recorded in this study is lower than that of *Cyperus esculentus* (Sabiu et al., 2017), *Adiantum caudatum* and *Celosis argentea* (Telagari et al., 2015) but higher than what has been reported for *Cissus cornifolia* (Chipiti et al., 2017).

The nature of Lineweaver-Burk plot is such that in an uncompetitive mode of inhibition, K_m and V_{max} are lowered or decreased in the presence of an inhibitor (Nelson, et al 2008). In this study, both extracts (LTE and SSE) demonstrated an uncompetitive inhibition on α-glucosidase enzyme as confirmed by the kinetic parameters computed. The decline in K_m and v_{max} values displayed by LTE and SSE could be due to the presence of some active ingredients that may be present in these plants extracts. Generally, an uncompetitive inhibitor binds at an allosteric site which is distinct from the enzyme's active site. Another characteristic feature of this type of inhibition is that, the inhibitor binds to only the enzyme–substrate (ES) complex. This allosteric interaction causes a conformational change in the globular structure of the enzyme thereby reducing the catalytic efficiency of the enzyme in converting substrate to product (Nelson et al., 2008). This implies that plant extracts investigated in this study possess active compounds capable of binding to allosteric sites on the ES (α-glucosidase-*p*NPG) complex and cause a reduction in the catalytic efficiency of α-glucosidase. This mechanism may have accounted for the decline in the enzyme's reaction velocity when inhibitors (LTE and SSE) were introduced.

Several important secondary metabolites are noted as contributing to therapeutic effectiveness in many plant natural products. Phytochemicals namely terpenoids, flavonoids, tannins, saponins and alkaloid have been reported to be present in methanolic extract of *Launaea taraxacifolia* leaves and also in ethanolic extract *Strychnos spinosa* leaves (Isa et al., 2014). This is not different from what has been observed in this study regarding these plants. There is evidence illustrating the inhibitory effects of phytochemicals such as flavonoids, on α-glucosidase enzyme (Wei et al., 2015; Zhang et al., 2015; Dendup, et al., 2014). It is clear from this study, that extracts of *Lauanea taraxacifola* and *Strychnos spinosa* leaf displayed effective inhibition against *α*-glucosidase enzyme and this may be associated with the synergistic effect of these phytochemical constituents present in the leaf extracts. The inhibitory property of *Lauanea taraxacifola* and *Strychnos spinosa* leaves extracts could be explored to control postprandial hyperglycaemia in type-2 diabetes mellitus individuals.

5. Conclusion

Extracts of *Launaea taraxacifolia* and *Strychnos spinosa* leaves displayed effective *in vitro* inhibition against isolated intestinal *α*-glucosidase enzyme in an uncompetitive mode. The study also concludes that *Launaea taraxacifolia* and *Strychnos spinosa* contain bioactive compound that may be sources of lead compounds for *α*- glucosidase inhibitors.

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