



Evaluation of α -Amylase Inhibitory Potentials of *Sida acuta*, *Tithonia diversifolia* and *Chromolaena odorata* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author FKA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AA and OOO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the inhibitory effect of *Sida acuta*, *Tithonia diversifolia* and *Chromolaena odorata* leaf extracts on the activity of carbohydrate hydrolyzing enzyme (α -amylase) and to provide scientific validation of their folk use in diabetes treatment.

Study Design: The α -amylase inhibition assay was carried out as a measure of the anti-diabetic potentials of *Sida acuta*, *Tithonia diversifolia* and *Chromolaena odorata*.

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Methodology: Cold water, hot water and ethanolic extracts of *Sida acuta*, *Tithonia diversifolia* and *Chromolaena odorata* were obtained. Standard method was employed in the α -amylase inhibitory assays (3, 5-dinitrosalicylic acid (DNSA) method).

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Results: The results revealed that the extracts had a dose dependent prevention of digestion of carbohydrates by inhibiting α -amylase. Ethanolic extracts of *T. diversifolia*, *C. odorata* and *S. acuta* gave the highest inhibitory activities against α -amylase with 41.02% (IC₅₀ 0.754 mg/ml), 43.67% (IC₅₀ 0.619 mg/ml) and 45.72% (IC₅₀ 0.604 mg/ml) maximum inhibition at a concentration of 500 μ g/ml respectively.

Conclusion: It can be concluded from this study that extracts of these plants possessed great potential as anti-diabetic agents by inhibiting α -amylase. The presence of phytochemicals like flavonoids, saponins, and tannins might have contributed greatly to the inhibitory activities exhibited by these plant extracts.

Keywords: *Diabetes mellitus*; α -amylase inhibition; *Sida acuta*; *Tithonia diversifolia*; *Chromolaena odorata*.

1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder which is characterized by chronic hyperglycemia (elevated fasting blood glucose level) resulting from the body's inability to produce or use insulin [1]. The control of hyperglycemia is important in the treatment of Type I and II diabetes because acute and chronic complications occur when the blood glucose concentration is not in the normal range (80-120 mg/dl) [2]. α -amylase is one of the main enzymes responsible for the breakdown of starch to more simple sugars. α -amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes [3]. Currently available therapeutic drugs like biguanides and sulphonamides have been shown to impose side effects like secondary renal failure, when used for longer periods [4]. Therefore, alternative sources of these medicines are being sourced from natural sources due to their good pharmacological properties, fewer side effects, and low cost [5].

Sida acuta is a malvaceous weed that frequently dominates improved pastures, waste and busy roadsides [6]. It is commonly called stubborn grass. It is used orally for asthma, fever, aches, pains, ulcers, antihelmintic medications as well as for venereal diseases. Its roots are used as diuretic, astringent, stomachic, febrifuge, and demulcent and seeds are applied as laxative, aphrodisiac, and demulcent, recommended in cystitis, colic, gonorrhoea, mental or nervous strain and piles [7]. The leaves are considered to possess demulcent, diuretic, antihelmintic and wound healing properties and also used in rheumatic infections. The juice of the leaves are

boiled in oil and applied to testicular swellings and in Elephantiasis and also leaf juice is used as a poultice for dandruff [8]. In Nigeria, *S. acuta* is used to treat malaria, ulcer, fever, gonorrhoea, abortion, breast cancer, poisoning, inflammation, stops bleeding, treatment of sores wounds, antipyretic and the infusion of the leaves is given to women in labour [9,10,11,12].

Tithonia diversifolia belongs to the family *Asteraceae*. It is a tropical herb or shrub cultivated in many countries of Africa, Asia, and South America for its multipurpose value. Its common name is sun flower. The leaf juice of *T. diversifolia* is used for vomiting and gastric disorders. In Nigeria, the decoctions of the various parts of *T. diversifolia* are used for the treatment of malaria, diabetes mellitus, sore throat, liver, menstrual pains and inflammation [13]. An oral decoction of the leaves and stems are used for the treatment of hepatitis in Taiwan and gastrointestinal disorders in Kenya and Thailand [14]. In Costa Rica, the dried leaves are applied externally on wounds [15] while in Cameroon; an infusion of the leaves is used for the treatment of measles [16]. In southern Thailand, leaves decoction of *T. diversifolia* was used to reduce hyperglycemic condition in diabetes patients, but it was not supported with laboratory data [17].

Chromolaena odorata (L) King and Robinson belongs to the family *Asteraceae*. It is a rapidly growing perennial herb and it is used as a traditional medicine in Indonesia, Thailand, Malaysia and parts of Africa including Nigeria. In Nigeria (Yoruba), it is locally called Akintola. The young leaves are crushed, and the resulting liquid has been used to treat skin wounds. In traditional medicine of Thailand, the plant is used for the treatment of wounds, rashes, diabetes, and as insect repellent. It has been studied to have antispasmodic, diuretic, hepatotropic,

astringent, anti-inflammatory, antihypertensive, antiprotozoal, antitrypanosomal, antibacterial and antifungal activities [18,19,20].

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *Tithonia diversifolia*, *Chromolaena odorata* and *Sida acuta* were obtained from Oye Town in Ekiti State, Nigeria. The plants were identified and authenticated at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2 Reagents and Chemicals

Starch and pancreatic α -amylase were products of Sigma-Adrich Co., St Louis, USA. Other chemicals and reagents used were of analytical grade and water used was glass distilled.

2.3 Preparation of Plant Extracts

The leaves of *S. acuta*, *T. diversifolia* and *C. odorata* were dried at room temperature and ground to powder. The powdered leaves were divided into three portions (500 g) and each portion was separately extracted with cold water, hot water and ethanol (1000 ml) as described by [21]. The resulting infusions were decanted, filtered and evaporated to dryness using a rotatory evaporator.

2.4 α -Amylase Inhibitory Assay

The inhibition assay was carried out using the 3, 5-dinitrosalicylic acid (DNSA) method as reported by [22]. The total assay mixture composed of 1000 μ l of 0.02 M Sodium phosphate buffer (pH 6.9 containing 6 mM Sodium chloride), 1000 μ l (0.04 units of pancreatic α -amylase solution) and 400 μ l extracts at various concentration ranging from 100-500 μ g/ml (w/v). After pre incubation at 37°C for 10 min, 1000 μ l of 1% (w/v) starch solution was added to each tube and incubated at 37°C for additional 15 min. The reaction was terminated with 1.0 ml DNSA reagent, and placed in boiling water for 5 min after which it was cooled to room temperature, and the absorbance was measured at 540 nm using vis spectrum lab S23A. The control did not contain any plant extract and represented 100% enzyme activity.

The % inhibition of α -amylase was calculated as follows:

Inhibitory activity (%)

$$= \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})}{\text{Absorbance}_{\text{control}}} \times 100$$

The IC₅₀ values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentrations of the plant extracts. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean \pm SE for triplicate determinations.

3. RESULTS AND DISCUSSION

Amylases (1-4-glucan-4-glucanohydrolases EC 3.2.1.1) are hydrolases that catalyse the hydrolysis of glycosidic bonds. They are probably the first group of enzymes to be discovered and currently of great industrial importance. α -amylases are involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. Pancreatic α -amylase as a key enzyme in the digestive system, is involved in the breakdown of starch into disaccharides and oligosaccharides and finally liberating glucose which is later absorbed into the blood circulation. Inhibition of α -amylase would diminish the breakdown of starch in the gastro-intestinal tract thus reducing the postprandial hyperglycemia level. It has been reported that inhibitors usually do not alter the total amount of carbohydrate absorbed and therefore do not cause any net nutritional caloric loss although they slow down carbohydrate digestion [23].

All the three plant extracts exhibited potent inhibition of α -amylase activity. The extracts clearly showed a dose dependent trend in their α -amylase inhibition. Dose-dependent inhibitory effects of plant extracts on α -amylase have been reported [24]. The ethanolic extract of *S. acuta* gave the highest percentage inhibition of α -amylase followed by its cold water extract and the hot water extract at 500 μ g/ml. Their α -amylase inhibitory order was EtOH

extract > cold water extract > hot water extract (45.92%, 19.55% and 16.40% respectively) Table 1, Fig. 1.

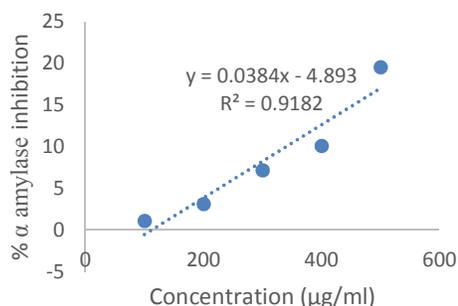
(43.67%, 16.63% and 8.09% respectively) Table 3, Fig. 3.

This trend was also observed with *T. diversifolia*: EtOH extract > cold water extract > hot water extract (41.02%, 15.71%, 13.13% respectively) Table 2, Fig. 2 and *C. odorata*: EtOH extract > cold water extract > hot water extract

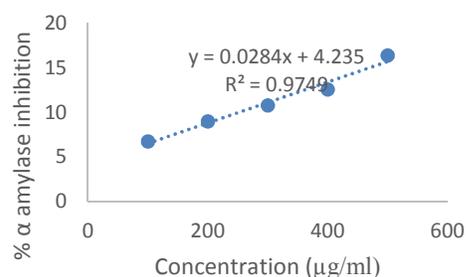
The same trend of α -amylase inhibition was also obtained for their IC_{50} (IC_{50} is the minimum concentration that caused 50% inhibition of alpha amylase) values with the ethanolic extract having the least IC_{50} followed by the cold water extract and then the hot water extract (Tables 1- 3).

Table 1. α -Amylase inhibitory activities and IC_{50} values of different Concentrations of *S. acuta*

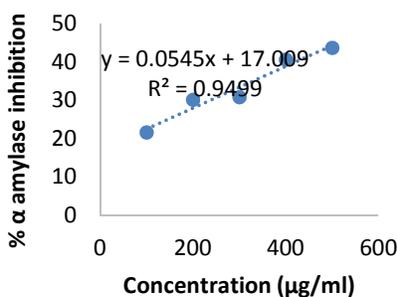
Extracts	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	IC_{50} (mg/ml)
Cold	500	19.55	1.430 ± 0.07
	400	10.11	
	300	7.19	
	200	3.15	
	100	1.12	
Hot	500	16.40	1.611 ± 0.05
	400	12.58	
	300	10.79	
	200	8.99	
	100	6.74	
Ethanol	500	45.92	0.604 ± 0.03
	400	41.77	
	300	32.11	
	200	31.22	
	100	29.43	



Cold water extract of *S. acuta*



Hot water extract of *S. acuta*



Ethanolic extract of *S. acuta*

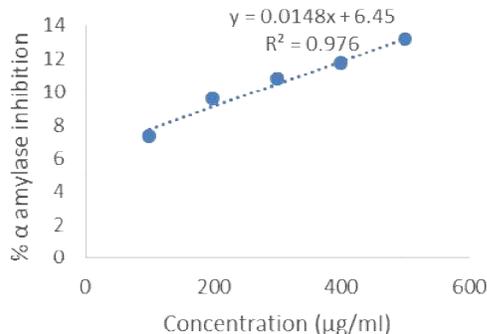
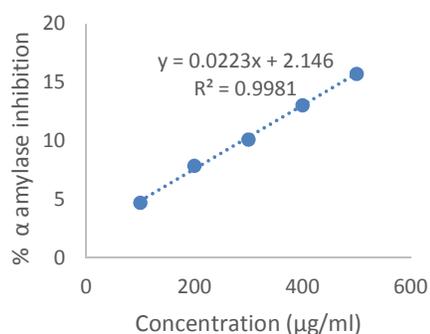
Fig. 1. Plot of percentage α -amylase inhibitory activities against different concentrations of *S. acuta*

Table 2. α -Amylase inhibitory activities and IC₅₀ value of different concentrations of *T. diversifolia*

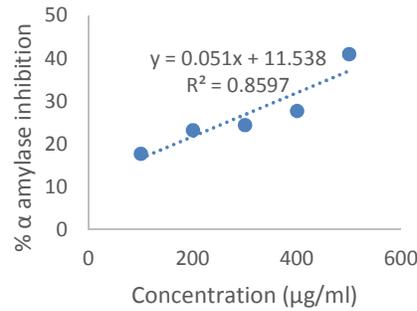
Extracts	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	IC ₅₀ (mg/ml)
Cold	500	15.71	2.146 \pm 0.09
	400	13.03	
	300	10.11	
	200	7.87	
	100	4.72	
Hot	500	13.13	2.943 \pm 0.12
	400	11.69	
	300	10.79	
	200	9.53	
	100	7.34	
Ethanol	500	41.02	0.754 \pm 0.01
	400	27.76	
	300	24.46	
	200	23.26	
	100	17.75	

Table 3. α -Amylase inhibitory activities and IC₅₀ values of different Concentrations of *C. odorata*

Extracts	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	IC ₅₀ (mg/ml)
Cold	500	16.63	2.146 \pm 0.13
	400	11.91	
	300	9.89	
	200	9.66	
	100	7.19	
Hot	500	8.09	6.110 \pm 0.05
	400	7.87	
	300	7.42	
	200	6.74	
	100	4.94	
Ethanol	500	43.67	0.629 \pm 0.02
	400	40.61	
	300	30.82	
	200	30.14	
	100	21.63	

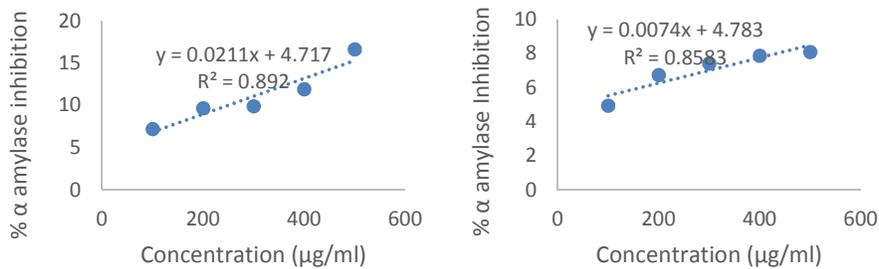


Cold water extract of *T. diversifolia* Hot water extract of *T. diversifolia*



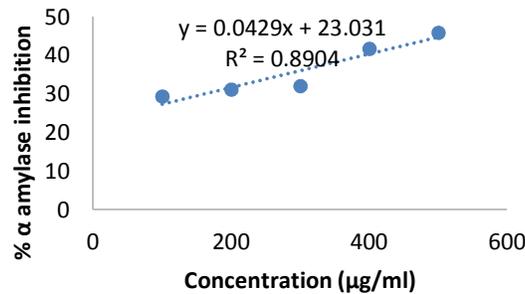
Ethanolic extract of *T. diversifolia*

Fig. 2. Plot of percentage α -amylase inhibitory activities against different concentrations of *T. diversifolia*



Cold water extract of *C. odorata*

Hot water extract of *C. odorata*



Ethanolic extract of *C. odorata*

Fig. 3. Plot of percentage α -amylase inhibitory activities against different concentrations of *C. odorata*

With the three different modes of extraction employed in this study, it was evident that these three plants possessed α-amylase inhibitory activities. Both the aqueous and ethanolic extracts of plants have been reported to possess α-amylase inhibitory activities [21,25,26,27]. In this study, the ethanolic extracts of all the three plants gave the highest percentage α-amylase inhibition. This suggests that ethanol could possibly be the best solvent for extracting active principles with α-amylase inhibitory potential from

the plants. There was no significant difference in the percentage α-amylase inhibition when hot and cold water were used as the extracting solvents (Tables 1-3).

α-Amylase inhibitors with activity against mammalian forms of the enzyme are present in many plants [27] and it was suggested that they were developed by plants in order to strengthen their defense against predators [28]. The aflavins and catechins present in green and black teas

have been reported to inhibit α -amylase and α -glucosidase activity as well as retard starch digestion in an *in vitro* model [29]. α -amylase inhibitors are also present in grains, including wheat and rice [28]. α -amylase inhibitors such as acarbose and miglitol are currently in clinical use to prevent the digestion of carbohydrates and provide short-term glycemic control. The drawback of such inhibitors is their non-specificity in targeting different glycosidases [30] thereby producing serious side effects that limit their use as therapeutic drugs [31]. Therefore, extracts from medicinal plants are investigated for their potential as antidiabetic drugs.

Investigations have shown that the use of herbs by diabetic patients is a common practice worldwide and many herbal extracts have been reported for their anti-diabetic activities [31,32, 33,34,35]. Over 400 traditional plants with antidiabetic effects have been recorded but very few of these traditional plants have received proper scientific or medical investigation [36]. *S. acuta*, *T. diversifolia* and *C. odorata* have locally been employed in the treatment of diabetes [37]. Aqueous and methanolic extract of *S. acuta* have been reported to decrease the blood glucose in alloxan induced diabetic rats and increases the tolerance for glucose in glucose fed normal rats similar to glibenclamide, an anti-hyperglycemic agents [38]. Insulin-like impact, possibly through high glucose utilization or upgrading the sensitivity of β -cells to glucose, has been suggested as possible mechanism of its reported antihyperglycemic activity [34].

The hypoglycemic effects of *T. diversifolia* have also been reported [37]. This effect have been attributed to its phenolic content that correlated to its antioxidant capacity which have been speculated to play a role as free radical scavengers in diabetic patients [39,40] by preventing macrovascular and microvascular complications [41].

C. odorata have also been reported to lower glucose levels when administered to rats [42]. This hypoglycemic effect might have been achieved by increasing insulin secretion and peripheral utilization of glucose in diabetic rats, inhibition of endogenous glucose production, inhibition of intestinal glucose absorption and/or regenerating existing β cells [43]. These activities have been attributed to its antioxidant properties which correlated strongly to its phenolic contents [27]. Plant secondary metabolites with antioxidant potential have shown ameliorative effect on oxidative stress induced damage in

diabetes [44]. Phenolic compounds are known to inhibit the activities of carbohydrate-hydrolyzing enzymes due to their ability to bind with proteins [45,27]. Also, different flavonoids have been reported to possess high inhibitory potential towards α -amylase enzyme activity [46,40, 28,35]. The findings from this work suggest α -amylase inhibition as a possible mechanism of the anti-hyperglycemic effect of *S. acuta*, *T. diversifolia* and *C. odorata* and further support the use of these plants in diabetes treatment.

4. CONCLUSION

It can be concluded that, the extracts of these three plants possessed α -amylase inhibitory potential, with the ethanolic extracts of the plants displaying the most effective inhibition. Since the global scenario is now changing towards the use of nontoxic plant product with increased potency and lesser adverse effects than existing drugs, development of modern drugs from *S. acuta*, *T. diversifolia* and *C. odorata* for the treatment of diabetes should be encouraged. These plants hold tremendous potential for pharmaceutical values.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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