

Comparative α -amylase and α -glucosidase inhibitory potency and mode of inhibition of aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red'

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ABSTRACT

The objective of this study was to investigate comparatively the inhibitory potency and mode of inhibition of aqueous leaf extracts of *Acalypha wilkesiana* 'green' (*A.w._g*) and *Acalypha wilkesiana* 'red' (*A.w._r*) on alpha glucosidase from *Saccharomyces cerevisiae* and alpha amylase from *Aspergillus oryzae* with a view to establishing the better antidiabetic plant. This study employed para-nitrophenylglucopyranoside (p -NPG) and starch as substrates while Acarbose was used as reference. We also qualitatively screened for important phytochemicals as well as the phenolic contents of the extracts. The extracts contained alkaloid, flavonoid, saponin, tannin, steroid, carotenoid and terpenoid while anthraquinone was absent. Aqueous extract of *A.w._r* had the higher total phenolics (total phenol: 35.75±3.39mgGAE/g and total flavonoid: 350.63±5.01mgQE/g). *A.w._r* expressed the higher inhibitory effect against alpha glucosidase and alpha amylase with IC_{50} values of 85.6 μ g/ml and 75.6 μ g/ml respectively in a near competitive fashion while the *A.w._g* exhibited mixed non-competitive inhibition of alpha amylase and alpha glucosidase. We can therefore deduce from this study that aqueous extract of *Acalypha wilkesiana* 'red' is a more potent inhibitor of alpha amylase and alpha glucosidase.

Keywords: α -glucosidase; α -amylase; Total phenol; Flavonoids

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INTRODUCTION

Acalypha wilkesiana Muell. Arg. otherwise known as Jacob's coat, match me if you can, fire dragon or copper leaf belong to the family of Euphorbiaceae and to the genus *Acalypha* which consist of more than 500 species [1]. *Acalypha wilkesiana* is native to Fiji and near islands of south pacific. They are globally distributed with substantial occurrence in the tropics of Asia, America and Africa [2]. It has 4 – 8 inch heart shaped leaves presented with various mottled color combinations ranging from green with edged white patches to coppery green to copper, red or even pink-patched leaves, hence its ornamental feature. It is generally found across the regions of West Africa and widely spread in southern Nigeria [3]. The folkloric use of *Acalypha wilkesiana* has been established by several reports; [4] reported the antimicrobial activity of the plant, [5] reported the antioxidant activity of the plant. [6] reported antimalarial activity. [7] reported the usefulness of *Acalypha wilkesiana* in the management of diabetes. The findings of [8] were re-affirmed by [9]. These antidiabetic establishments were further strengthened by the in-vitro investigations [10], where they revealed the inhibitory potency of *Acalypha wilkesiana* against α -glucosidase.

α -Glucosidase and α -amylase are important enzymes involved in the breakdown of large carbohydrate molecules and consequent increase in concentration of blood glucose especially after a carbohydrate-rich meal, whose uncontrolled actions can pose significant danger to the health of type 2 diabetics and borderline patients [11]. Dietary carbohydrate are composed of glucans; amylose with linear chain of α -1,4-glycosidic bonds and amylopectin with α -1,4 and α -1,6-glycosidic linkages [12]. Salivary and pancreatic amylases hydrolyze the α -1,4-glycosidic bonds in starch and shorter oligomers giving rise to maltose, maltotriose and dextrin (mixture of D-glucose polymers units linked by α -1,4 and α -1,6-linkages [13]. Products of hydrolysis by α -amylases are further hydrolysed into monosaccharides (glucose, fructose or galactose) by small intestinal membrane bound α -glucosidases such as Sucrase-isomaltase and Maltose glucoamylase hydrolyzing α -1,4-linkages to yield α -D-glucose. The N-terminal of Sucrase-isomaltase (NtSI) and C-terminal domain Sucrase-isomaltase (CtSI) cleave α -1,6-glycosidic linkage of amylopectin and α -1,2-linkages of sucrose with all cleavages releasing glucose end product which is further subjected to energy-yielding metabolisms. This chain of breakdown continues so long polysaccharides are available

within the body system especially following a carbohydrate rich meal.

In a non-insulin dependent diabetic condition when the body is unresponsive to insulin, emanating from impaired stimulation of physiological responses necessary to facilitate trafficking of GLUT-4 from storage vesicles to the plasma membranes of skeletal muscle and adipose tissue consequently leading to impaired uptake of glucose from circulation, blood glucose concentration rises and hyperglycemia ensues [14]. The management of blood glucose level is one of the major target goals in the management of type 2-diabetes which can be achieved through regular exercise, healthy dieting and use of α -amylase and α -glucosidase inhibitors [15] among others. Inhibitors are proven agents of glucose level control. Synthetic inhibitors such as voglibose, acarbose, miglitol etc are not without side effects, a more reason why the search for plant based inhibitors is still a scientific interest [16, 17]. Acarbose, a microbial pseudotetracosaccharide is a potent inhibitor of α -glucosidase and α -amylase, clinically being useful especially in synergy with other antidiabetic agents to achieve suppression of post prandial hyperglycemia [18, 19], however the use of Acarbose is reportedly associated with a number of side effects such as diarrhea and abdominal discomfort [20]. Voglibose, an α -glucosidase inhibitor exerting its antidiabetic actions by reversible inhibition of the enzyme as well as facilitating the increased release glucagon-like-peptide-1 (an insulinotropic hormone) which has inhibitory action on glucagon, thus limiting fasting glucose level [21] had however been documented to have adverse reactions such as nausea and abdominal pain among others [22]. Natural product inhibitors, as opposed to voglibose and acarbose, are reported to possess significant inhibition towards α -glucosidase and even α -amylase with minimal or no side effects [23]. The medicinal plants *Acalypha wilkesiana*, as evident by reports [24] have established antidiabetic properties, however studies on the comparative potency of the red and green colored *Acalypha wilkesiana* with respect to inhibition of α -glucosidase and α -amylase and their modes of inhibition, to the best of our knowledge are absent in literature or scanty if available. Hence, the objective of this study.

MATERIALS AND METHODS

Plant materials

Our plant materials, leaves of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red' were obtained from same location 'Olusegun Agagu University of Science and Technology (OAUSTECH)' mega school campus, Okitipupa local government area of Ondo state, Nigeria in February, 2022 and authentication was done in plant biology department of OAUSTECH.

Chemicals and reagents

Alpha glucosidase from *Saccharomyces cerevisiae*,

alpha amylase from *Aspergillus oryzae*, para-nitrophenylglucopyranoside (p -NPG), acarbose, starch, Folin-Ciocalteu reagent, gallic acid and quercetin were gotten from Sigma – Aldrich (St. Louis, MO. USA). Other reagents and chemicals used were of analytical grade and water was distilled.

Preparation of plant materials

Leaves of plants were cleaned via washing and spread for 14 days under shade to air-dry and subsequently ground to powder. 350g was soaked in 1 liter of distilled water for 48 hours. Extract was dried using rotary evaporator and yield was stored at 4°C prior to analysis.

Alpha amylase inhibition assay

We adopted the assay procedure of McCue as modified by [25]. Briefly 250 μ l of different concentrations of the extract (20, 40, 60, 80 and 100 μ g/ml) was added to same quantity of 0.02M sodium phosphate buffer of pH 6.9 containing α -amylase solution (0.5mg/ml) in a test tube. Preincubation at 25°C for 10 mins was done, followed by addition of 250 μ l of 1% starch in 0.02M sodium phosphate buffer (pH 6.9) at timed interval and incubated further for 10 mins at room temperature. 0.5ml of dinitrosalicylic acid (DNSA) was introduced in order to terminate the reaction. All tubes were incubated at boiling temperature and then cooled to room temperature followed by addition of 5ml of distilled water to dilute reaction mixtures. 'Control' contained same content except that extract was replaced with distilled water. Absorbance measurement was carried out at 540nm using spectrophotometer. Result of inhibition was expressed in percentage and calculated using the formula;

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} * 100$$

Concentration of extract inhibiting half population of enzyme (IC₅₀) was determined graphically.

Mode of inhibition of alpha amylase

Assay of mode of inhibition of α -amylase by aqueous extract of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red' was carried out by a modified method of [26]. 250 μ l of the extracts was added to 250 μ l of α -amylase solution and preincubated at 25°C for 10 minutes in one set of test tubes. α -Amylase added to 250 μ l of pH 6.9 phosphate buffer was also preincubated in a different set of test tubes. To start the reaction, 250 μ l of varying and increasing concentration of starch solution (1.0 – 5.0mM) were added to the two sets of reaction tubes. All reaction mixtures were incubated at room temperature for 10 mins. 0.5ml of DNSA was added to halt the reactions and boiling for 5 minutes followed. Quantity of released reducing sugar was measure by spectrophotometry and extrapolated from standard maltose curve and then converted to reaction velocities (V). A Lineweaver Burk plot or double reciprocal plot of the inverse of reaction velocities (1/V) versus inverse of substrate concentrations (1/[S]) was generated and the mode of inhibition of α -amylase by the aqueous extracts of both plants were thus determined (Nelson and Cox, 2008) [27].

Alpha glucosidase inhibition assay

The assay protocol [28] was adopted here with slight modification as reported [29]. 250 μ l of varying concentrations (1.0 – 5.0mM) of the extracts were introduced into test tubes, 50 μ l of alpha glucosidase (1U/ml) from *Saccharomyces cerevisiae* was added into all the test tubes. 30 μ l of 5.0 mM para-nitrophenyl- α -D-glucopyranoside (p -NPG) already prepared in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. Incubation of reaction mixtures was done at 37°C for 60 minutes and stoppage of reaction by adding 1ml of 0.1M sodium carbonate (Na_2CO_3). Enzyme (α -glucosidase) activity was determined by measuring the released yellow colored p-nitrophenol from PNPg at a wavelength of 405nm using spectrophotometer. Results were presented as percentage of blank control.

Inhibition (%) = $\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} * 100$
Concentration of extracts inhibiting 50% activity of enzyme (IC_{50}) was determined graphically.

Mode of inhibition of alpha glucosidase

The α -glucosidase inhibitory mode of the extracts was carried out as described [30]. Briefly two different sets of test tubes were prepared. 50 μ l of extracts' concentrations (0, 85.6 & 171.2 μ g/ml for the $A.w_r$ and 0, 106.8 & 213.6 μ g/ml for the $A.w_g$) were preincubated with 100 μ l of α -glucosidase solution at 25°C for 10 mins in one set of test tubes while in the other set, α -glucosidase was preincubated with 50 μ l of pH 6.9 phosphate buffer. 50 μ l of p -NPG at increasing concentrations (1.0 – 5.0mM) was then introduced into the test tubes to begin reactions. Mixtures were incubated at room temperature for 10 mins after which 0.5ml of Na_2CO_3 was added to stop the reaction. Using paranitrophenol standard curve, amount of reducing sugar released was determined by spectrophotometry and converted to reaction velocities. A line weaver Burk plot of inverse of velocities (1/V) against inverse of substrate concentrations (1/[S]) was plotted and mode of inhibition of α -glucosidase by extracts determined (Nelson and Cox, 2008).

Determination of total phenol

The total phenol contents of the extracts was estimated using the Folin-Ciocalteu colorimetric method of [31], a method which relies on electron transfer from phenolic compounds in alkaline medium giving rise to phosphotungstic/phosphomolybdenum complex indicated by a blue coloration where the depth of color is directly proportional to the concentration of phenolics. 200 μ l of extract was mixed with 1000 μ l Folin-Ciocalteu reagent. After 4 mins, 1000 μ l of 7.5% sodium carbonate (Na_2CO_3) was introduced. Mixture was then incubated at 25°C for 2 hours. Absorbance was measured at 760nm and total polyphenol in the extracts was expressed as mg of gallic acid equivalent per gram of extract (mgGAE/g).

Determination of total flavonoid content

We adopted the Aluminium chloride (AlCl_3) reagent

method as described [32]. Briefly, 1 ml of extract was mixed with 1 ml of AlCl_3 (2%) in methanol. Reaction mixtures were incubated at 25°C for 10 mins and immediately followed by absorbance measurement at 430nm using spectrophotometer. Result was expressed as mg of quercetin equivalent per gram of extract (mgQE/g).

Phytochemical screening

Investigations for the presence of phytochemicals in the aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red' were done by the standard procedures of Sofowora (1993) and Trease and Evans (2002).

Statistical analysis

Assays were carried out in triplicates and results were expressed as mean \pm standard deviation (SD). Analysis of data was carried out using graph pad prism (graphpad software Inc. San Diego, USA) and statistical difference between groups was done by one way analysis of variance (ANOVA) and Duncan multiple range test. Values were considered significant at $p < 0.05$.

DISCUSSION

Physiological activities necessary to mobilize GLUT-4 carrier vesicles to cell membranes of myocytes and adipocytes for internalization of GLUT-4 molecules and subsequent insulin-regulated uptake of glucose for metabolisms may be distorted as a result of unresponsiveness to insulin, defects in insulin actions and/or insulin secretory processes, thus resulting in persistent elevated glucose level [33]. Post prandial hyperglycemia (PPHG) is characteristic of type-2-diabetes and risk factor for macrovascular and microvascular complications [34]. It's close monitoring and control is critical in the clinical management of diabetes and related complications. Among a number of management strategies available in literature, the use of inhibitors of α -amylase and α -glucosidase is key, as inhibitors are understood to inhibit or delay the degradation of glucose polymers in the gastrointestinal environment and limit post prandial glucose excursion in type-2-diabetes [35]. The unbearable side effects associated with the use of synthetic drugs (α -glucosidase and α -amylase inhibitors) triggered the search for medicinal plant-based inhibitors with promising level of potency and less side effects.

In this study, we evaluated and compared the inhibitory potency and mode of inhibition of aqueous extracts of *Acalypha wilkesiana* 'green' ($A.w_g$) and *Acalypha wilkesiana* 'red' ($A.w_r$) against α -glucosidase and α -amylase. Across the tested concentrations (20 – 100 μ g/ml), the aqueous extract of $A.w_r$ showed higher inhibition (in percentage) towards α -amylase, although statistically insignificant ($p < 0.05$) when compared to $A.w_g$ except at 80 μ g/ml and 100 μ g/ml. Values of extracts are significantly different ($p < 0.05$) from that of acarbose at all concentrations with the exemption of 80 μ g/ml where $A.w_r$ favorably compares with the standard (Fig.1.)

$A.w_r$ also showed promising inhibition of α -glucosidase and values were significantly higher ($p < 0.05$) than

those of *A.w.g* except at 20µg/ml and 60µg/ml. However, inhibition values of the two extracts were significantly lower ($p < 0.05$) when compared to acarbose with the exception of 20µg/ml, in which case *A.w.r* favorably compares with acarbose (Fig.2.)

The potency of the extracts was further ascertained by determining the IC_{50} values of the inhibition of α -amylase and α -glucosidase. For both enzymes, *A.w.r* showed higher inhibitory potency (with IC_{50} values of $76.11 \pm 2.23 \mu\text{g/ml}$ and $85.59 \pm 2.34 \mu\text{g/ml}$ respectively against α -amylase and α -glucosidase) than *A.w.g* (Tabs. 1-3.)

As for the modes of inhibition, from the line weaver Burk plot generated. *A.w.r* displayed a near competitive mode of inhibition against α -amylase and α -glucosidase (Figs. 3. and 4.), which is an indication that its active components compete with the substrate for the active site on both enzymes thereby inhibiting the degradation of large glucans. In the case of *A.w.g*, extract displayed mixed non-competitive mode of inhibition (Figs. 3. and 4.),

suggesting that its active components do not compete with the substrate for the active site on the enzymes; rather it binds to other sites on the enzymes to limit the breakdown of carbohydrate.

The significantly higher ($p < 0.05$) values of acarbose than the extracts is an indication of mild potency of the extracts against the enzymes, which is in agreement with previous reports that excessive inhibition of pancreatic α -amylase could trigger abnormal bacterial fermentation of undigested carbohydrates, as such mild inhibition, offered by most plant derived inhibitors is well appreciable. The inhibitory effects of these extracts could be a reflection of their total phenolic contents as their IC_{50} values directly correlate with their phenolic contents. The *A.w.r* had higher total phenol and flavonoid values (Tab.2.) Phenolic compounds are reportedly able to inhibit carbohydrate degrading enzymes due to their protein-binding properties. Our finding is thus in conformity with other reports that potential inhibitors of α -glucosidase and α -amylase belong

Fig.1. α -amylase inhibitory effect of aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red' (Values are expressed as mean \pm SD, n = 3). Means with different superscript at same concentration are significantly different ($p < 0.05$)

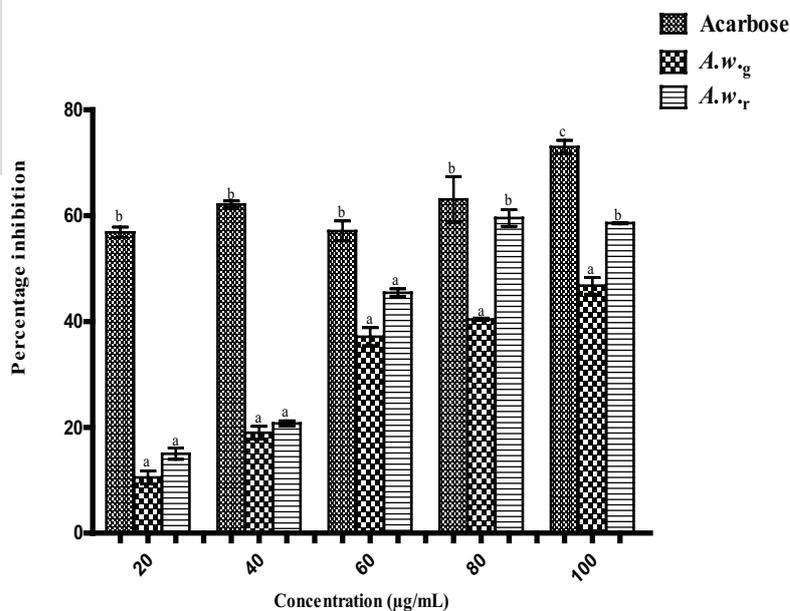
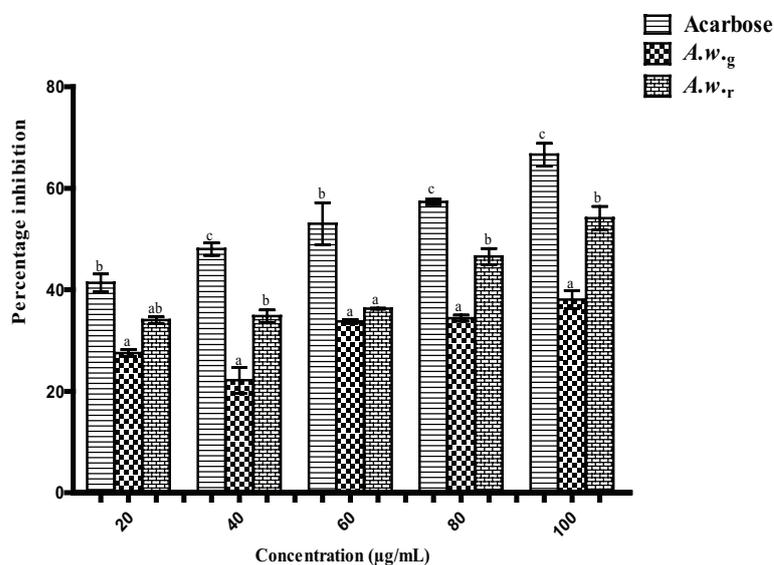


Fig.2. α -glucosidase inhibitory effect of aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red'. (Values are expressed as mean \pm SD, n = 3). Means with different superscript at same concentration are significantly different ($p < 0.05$).



Tab.1. Phytochemicals in aqueous extracts of *Acalypha wilkesiana* 'green' (*A.w.g*) and *Acalypha wilkesiana* 'red' (*A.w.r*)

Extract Phytochemicals	<i>A.w.g</i>	<i>A.w.r</i>
Alkaloid	+	+
Flavonoid	+	+
Steroid	+	+
Tannin	+	+
Saponin	+	+
Anthraquinone	-	-
Carotenoid	+	+
Cardiac glycoside	-	+
Terpenoid	+	+

+ = Present, - = Absent

Tab.2. Total phenol and flavonoid contents of aqueous leaf extracts of *A.w.g* and *A.w.r*

Extracts	Total Phenol (mgGAE/g)	Total flavonoid (mgQE/g)
<i>A.w.g</i>	21.61 ± 1.26	247.02 ± 9.10
<i>A.w.r</i>	35.75 ± 3.99	350.63 ± 5.01

Values are expressed as mean ± standard deviation – SD. (n = 3)

Tab.3. IC₅₀ values for α -amylase and α -glucosidase inhibition of aqueous extracts of *Acalypha wilkesiana* 'green', and *Acalypha wilkesiana* 'red'.

Samples	IC ₅₀ (μ g/ml)	
	α -amylase	α -glucosidase
Acarbose	46.33 ± 1.20 ^a	60.50 ± 2.52 ^a
<i>A.w.g</i>	102.53 ± 2.71 ^c	106.81 ± 2.92 ^c
<i>A.w.r</i>	76.11 ± 2.23 ^b	85.59 ± 2.34 ^b

The values are expressed as means ± SD of triplicate tests. Means on same column sharing different superscripts are significantly different ($P < 0.05$).

Fig.3. Lineweaver-Burk plot for inhibition of α -amylase by aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red'.

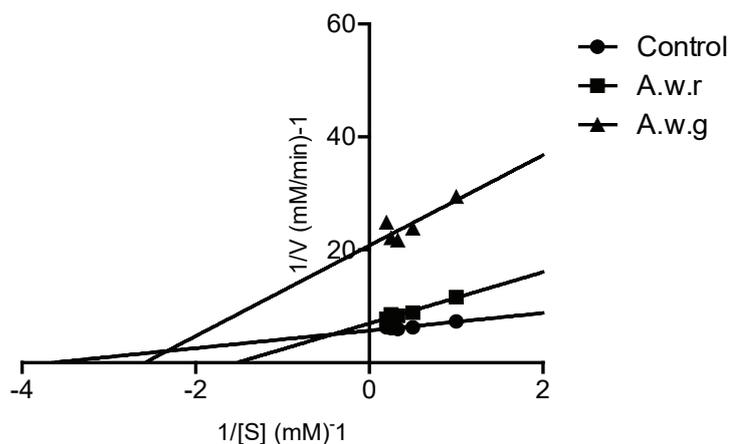
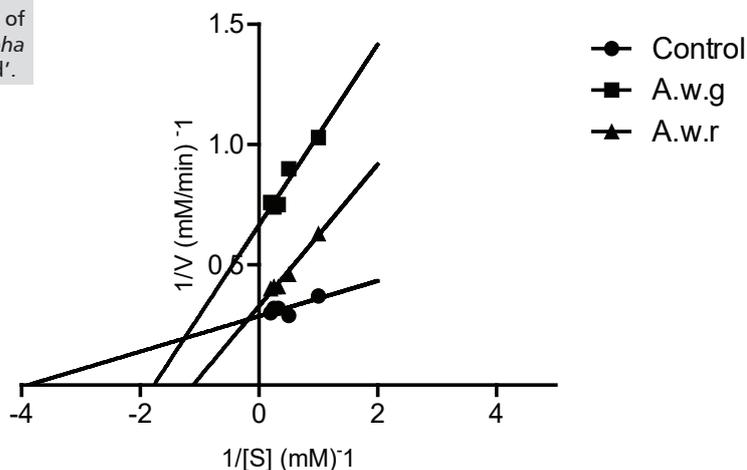


Fig.4. Lineweaver-Burk plot for inhibition of α -glucosidase by aqueous leaf extracts of *Acalypha wilkesiana* 'green' and *Acalypha wilkesiana* 'red'.



to the flavonoid class.

The presence of other phytochemicals such as terpenoid, saponin, tannin etc in the extracts of *A.w._r* and *A.w._g* (Tab.1.) could have also contributed to their inhibitory properties which is in line with the reports.

CONCLUSION

From this study, we can conclude that aqueous extracts of *A.w._r* and *A.w._g* have antidiabetic properties, conforming

to the reports of Ikewuchi and Ikewuchi (2010). The α -glucosidase and α -amylase inhibitory activities might be due to the presence of important phytochemicals such as flavonoid, saponin, terpenoid etc acting synergistically. We could also conclude that *A.w._r* had a higher inhibitory potency on α -amylase and α -glucosidase activities, as reflected by the IC_{50} values which could possibly be due to the higher phenolic contents detected in the extract.

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