

Oxidative stress mitigation and the inhibition of carbohydrate-hydrolysing, lipoxygenase (15-LOX), and acetylcholinesterase enzymes by methanolic crude extracts and partitioned fractions of *Rinorea dentata* (P Beauv.) O Ktze. leaf and stem

Ibukun O. Oresanya¹, Mubo A. Sonibare¹, Salman Adebayo², Fatai O. Balogun², Anofi O. T. Ashafa^{2*}

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

²Phytomedicine and Phytopharmacology research Group, Department of Plant Sciences, University of the Free State Qwaqwa, Free State, South Africa.

ARTICLE INFO

Received on: 16/10/2019
Accepted on: 18/03/2020
Available online: 04/07/2020

Key words:

Acetylcholinesterase, antihyperglycemic, lipoxygenase inhibition, medicinal application, *Rinorea dentata*.

ABSTRACT

Rinorea dentata (Violaceae) is a rare medicinal plant used in treating many diseases such as malaria, neurodegenerative diseases, body inflammation, and diabetes among others. The study investigated the *in vitro* pharmacological activities of crude extract and partitioned fractions from the leaf and stem parts. Antioxidant activity was evaluated using the ability of the crude extract and fractions to scavenge 2, 2-diphenyl-1-picrylhydrazyl radical and ferric reducing antioxidant power (FRAP). *In vitro* anti-inflammatory and anticholinesterase potential of the extracts, the inhibition of α -amylase (α -AL) and α -glucosidase (α -GSD), and kinetics of enzymes inhibition were evaluated by the standard methods. The ethyl acetate fractions (EAFs) of both leaf and stem gave the highest total phenolic content and the best antioxidant potential in the FRAP assay. For the anticholinesterase activity, crude extract of both leaf and stem had the highest inhibitory activity. Leaf EAF ($34.14 \pm 16.69 \mu\text{g/ml}$) had superior lipoxygenase inhibitory activity than quercetin ($54.75 \pm 17.07 \mu\text{g/ml}$) and better than the stem. The dichloromethane fraction had the best α -GSD and mild α -AL inhibitions with 204.00 ± 8.12 and $500.42 \pm 2.87 \mu\text{g/ml IC}_{50}$ values, respectively. *R. dentata* leaf extract going by the results of the investigation is a good source of antioxidant, anticholinesterase, anti-inflammatory, and antidiabetic agents for future drug development and sustainability.

INTRODUCTION

Rinorea dentata (P Beauv.) Kuntze. is a rare wild plant species, found in the tropics. It belongs to the family of Violaceae, growing majorly in the understory of humid or semi-deciduous forests and particularly abundant in Omo Forest Reserve, Ogun State, Southwestern Nigeria (Ojo, 2004). *R. dentata* is a shrub or small tree growing as tall as 10-m high dominating the lower forest strata, where exposure to sunlight becomes a challenge (Chuyong *et al.*, 2011). The common names of the plant include “iyokheze” (Edo), “oloborowo” (Yoruba), or “stone plant” (Burkill, 2000) and

used locally as a chewing stick for mouth cleaning. *R. dentata* is an important medicinal plant reported with various ethnomedicinal uses such as against neurodegenerative disorders, malaria, diabetes, headaches, diarrhea, and body inflammation (Attah *et al.*, 2016; Erhenhi and Obadoni, 2015; Jiofack, 2008). However, despite all these indigenous usages, the pharmacological reports on these ethnomedicinal claims are found wanting in the literature. This work was, therefore, designed to explore the pharmacological potentials of this important Nigerian medicinal plant.

Inflammation is an underlying element in the onset of many diseases such as diabetes, cancer, infections, obesity, and cardiovascular and even neurodegenerative diseases such as Alzheimer's (AD) (Corrado *et al.*, 2010; Crowson *et al.*, 2013). Certain non-steroidal anti-inflammatory drug on administration over a long period exhibits a decreased risk of AD. In fact, the report had found a link between the cholinergic system and

*Corresponding Author

Anofi Omotayo Tom Ashafa, Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State Qwaqwa, Phuthaditjhaba, South Africa. E-mail: ashafaot@ufs.ac.za

inflammation with acetylcholine playing a role in the release of cytokine (Borovikova *et al.*, 2000). Lipid-peroxidizing enzymes (lipoxygenase) required in the bioformation (from arachidonic acid) of leukotriene serve as an intermediary for inflammation and allergic responses. They are also involved in the induction of many diseases related to inflammation (Dobrian *et al.*, 2011) as they (lipoxygenases) trigger the introduction of molecular oxygen to unsaturated fatty acids (UFAs), such as linoleic and arachidonic acids, which are their common substrates (Porta and Rocha-Sosa, 2002). It is worthy of mention that there are four major isoenzymes of lipoxygenases including 5, 8, 12, and 15, with each isoform differing from one another based on the position of oxidation in the UFAs. Despite the major involvement of this enzyme in inflammation, their actions are similarly witnessed in the formation of inflammatory lipid mediators, such as leukotrienes and prostaglandins. Intriguingly, the inhibition of these enzymes is considered as the hallmark measure for disease prevention, particularly those linked to oxidative stress and inflammation and, perhaps, diabetes mellitus (Butterfield *et al.*, 2014; Radmark and Samuelson, 2007).

Diabetes mellitus (DM) is a chronic derangement attributed to partial or complete insulin deficiency arising from elevated glucose level in the blood (hyperglycemia) (Yadav *et al.*, 2008). The ability of any drug substance to salvage postprandial blood sugar levels (BGL) is considered as a very important step in early diabetes mellitus therapy and reduction in protracted vascular problems (Kwon *et al.*, 2008). Persistent starch hydrolysis by α -amylase (α -AL) of the pancreas and the subsequent glucose absorption by α -glucosidases (α -GSDs) found in the intestine lead to a continuous increase in BGL, termed as hyperglycemia in non-insulin-dependent diabetes (NIDD), individuals. Thus, a useful approach for NIDD management is a strong α -GSD and mild α -AL inhibition (Krentz and Bailey, 2005). Interestingly, the prevention of carbohydrate (CHO) absorption in the form of glucose after food uptake is a potent schedule or plan for alleviating postprandial hyperglycemia in patients with DM. It is exciting to also note that substances (inhibitors), which can hinder the activities of these enzymes by delaying CHO digestion, would bring about a reduction in the extent to which glucose is absorbed, consequently preventing post-prandial elevation of plasma glucose (Rabasa-Lhoret and Chiasson, 2004). The common examples of such inhibitors of α -AL and α -GSDs available in the pharmaceutical industry are acarbose, miglitol, and voglibose (Bailey, 2003) known to lower postprandial blood glucose after a starch load. These inhibitors exert their action by inhibiting the final process in CHO digestion [conversion of disaccharide to monosaccharide (glucose)], resulting in the reduced concentration of glucose absorbed into the blood circulation (Joubert *et al.*, 1990). Unfortunately, these drugs are not without side effects including bloating, abdominal distension, and flatulence. Hence, there is the need or search for alternative medicines from plants.

The aim of this study was to investigate the potentials of *R. dentata* crude extracts and fractions on the activities of 15-lipoxygenase (15-LOX), acetylcholinesterase (AChE), α -AL, and α -GSD with the hope of establishing potential treatments (from natural sources) that could come handy in managing

inflammatory-related, diabetes, and Alzheimer's diseases with the minimal or outright absence of side effects.

MATERIALS AND METHODS

Chemicals

Linoleic acid was purchased from Merck (Germany). Quercetin, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 15-lipoxygenase from *Glycine max*, Folin-Ciocalteu reagent, aluminum chloride, trolox, 2, 4, 6-tri(2-pyridyl)-s-triazine, 5,5'-dithio-bis-2-nitrobenzoic acid, eserine, acetylcholine iodide, electric eel AChE (C3389), acarbose, p-nitrophenyl glucopyranoside, α -AL, and α -GSD were also procured from Sigma-Aldrich, South Africa.

Plant material collection, authentication, and extraction

Rinorea dentata whole plant was collected from Omo Forest Reserve, Ogun, Nigeria, on February 10, 2016. The plant species was recognized and authenticated at the Forest Herbarium Ibadan (FHI) with voucher number FHI 111531. Voucher specimens were also deposited in the Department of Pharmacognosy Herbarium, University of Ibadan. The leaves were separated from the stem, air-dried, and powdered. The powdered leaves (1,000 g) and stem (2,000 g) were weighed and macerated in methanol for 4,320 minutes followed by another 1,440 minutes of extraction. The filtered extracts and filtrates were concentrated *in vacuo* resulting in the crude (leaf and stem) methanol extract.

The crude methanol extract (CME) of *R. dentata* leaf (RDL) (85.29 g) and *R. dentata* stem (RDS) (68.38 g) was dissolved in methanol-water (3:1) mixture and subsequently partitioned successively into *n*-hexane, dichloromethane, ethyl acetate, and *n*-Butanol fraction (NBF). All fractions obtained were concentrated and preserved at -4°C before assays.

Qualitative phytochemical analysis

The phytochemical analysis was performed on all the extracts and fractions for the presence of alkaloids, anthraquinones, flavonoids, phenolics, tannins, triterpenes, phytosterols, glycosides, and saponins adopting well-established qualitative procedures (Sofowora, 2006; Prashant *et al.*, 2011).

Determination (*in vitro*) of the study parameters

DPPH antioxidant and ferric reducing antioxidant power (FRAP) assays

The DPPH antioxidant activity of the samples and standard measured by their ability to scavenge the DPPH was evaluated using the method described by Susanti *et al.* (2007) and Ayoola *et al.* (2017). The FRAP of the samples was determined using the method of Musa *et al.* (2011) and Sonibare *et al.* (2018).

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The method of Khatoon *et al.* (2013) and Ayoola *et al.* (2017) was used to explore the TPC of all the extracts. The TPC expressed as milligram gallic acid equivalent (GAE)/g of extract was calculated from the gallic acid calibration curve ($y = 0.0099x + 0.1207$, $r^2 = 0.9228$). The colorimetric method involving the use

of aluminum chloride was adopted in assessing the TFC of the extracts (Ayoola *et al.*, 2017; Ebrahimzadeh *et al.*, 2009) and was expressed as milligram quercetin equivalent (QE)/g of extract, obtained from standard curves ($y = 0.0077x + 0.0884$, $r^2 = 0.9980$) prepared with 6.25–200 μg quercetin/ml.

Inhibition of 15-LOX enzyme and anticholinesterase assay

The inhibition of 15-LOX (enzyme) method was used to determine the anti-inflammatory activity of the extracts (Adebayo *et al.*, 2015), whereas AChE inhibition was determined using Ellman's colorimetric method modified by Elufioye *et al.* (2010). Different sample/control concentrations (0.1–1 mg/ml) were tested, whereas quercetin and eserine were used as the positive controls for the anti-inflammatory and anticholinesterase assays, respectively. The results were expressed as inhibitory concentration (IC_{50}), i.e., the concentration of extracts or controls that gave 50% enzyme inhibition.

Alpha-amylase and alpha-glucosidase enzymes inhibition, and kinetics of inhibitory assays

The α -amylase (α -AML) inhibition (McCue and Shetty, 2004) and α -GSD inhibitory potential (Kim *et al.*, 2005) of the extracts and fractions were determined based on cited standard methods. Different extracts or controls (7.81–250 $\mu\text{g}/\text{ml}$) were reacted with α -amylase from the porcine pancreas or α -GCD from *Saccharomyces cerevisiae*. Acarbose was used as the positive control. Moreover, the mode (kinetics) of α -AML and α -GCD inhibitions was examined by the method described by Ali *et al.* (2006). The kinetics of the inhibition of α -AML and α -GCD

by *R. dentata* (RD) was evaluated using Lineweaver and Burk equation (Lineweaver and Burk, 1934).

Statistical analysis

The experiment or assays were carried out three times, and the results were reported as mean \pm standard error of mean (SEM) followed by using a one-way analysis of variance. Duncan's new multiple range tests were used to compare the means. All statistical analyses were carried out by using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego, CA. The IC_{50} values for the anti-inflammatory, α -AL, and α -GSD inhibitory assays were calculated from the linear regression curve.

RESULTS

Extraction and fractionation yield

The leaf samples gave a better yield compared to the stem. The yield of the extracts was calculated and expressed as the weight of fraction/extract relative to the weight of the obtained crude/starting powdered sample. The percentage yield of the leaf ranged from 3.98 to 31.44, whereas the extraction yield for the stem ranged from 2.47% to 39.50%. The aqueous-methanol fraction (AMF) of both the studied parts of the plant gave the highest yields as shown in Table 1. Polar solvents were shown to produce high extraction yield.

Phytochemical screening

The qualitative phytochemical screening of the crude extract and fractions of the leaf and stem of *R. dentata* revealed the presence of alkaloids aside from the *n*-hexane fraction (NHF) of the leaf and NBF as well as NHF (stem). Saponin was present in all the extracts and fractions except CME of the stem. Phenols were present in the AMF, NBF, ethyl acetate fraction (EAF), and dichloromethane fraction (DCMF) of the leaf and EAF and DCMF fractions of the stem. While anthraquinone was absent in all the crude extracts and fractions, triterpenes and steroids were found in AMF of the leaf and stem and CME of the stem. Flavonoids were similarly found in the AMF, NBF, and EAF of the leaf and AMF, CME, and EAF of the stem. Tannins were detected only in the EAF of the leaf and the EAF and DCM of the stem (Table 2).

Table 1. Extraction yield and percentage yield of RDL and stem.

Extract/fractions	Leaf		Stem	
	Yield (g)	% Yield	Yield (g)	% Yield
CME	107.64	10.76	81.90	4.09
NHF	24.56	28.79	9.637	14.09
DCMF	3.40	3.98	12.21	17.86
EAF	3.44	4.03	1.68	2.47
NBF	12.83	15.04	3.49	5.11
AMF	26.82	31.44	27.01	39.50

Table 2. Phytochemical screening of the extract and fractions of RDL and stem.

Phytochemicals	Leaf						Stem					
	AMF	CME	NBF	EAF	DCM	NHF	AMF	CME	NBF	EAF	DCM	NHF
Alkaloids	++	+	+	+	+	-	-	++	-	++	++	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	++	-	+	++	-	-	+	+	-	-	-	-
Phenols	+	-	++	++	+	-	-	-	-	++	+	-
Tannins	-	-	-	++	-	-	-	-	-	++	++	-
Triterpenes	++	-	-	-	-	-	+	++	-	-	-	-
Steroids	++	-	-	-	-	-	++	++	-	-	-	-
Saponins	++	+	+	++	+	++	++	-	+	++	++	+
Glycoside	-	-	-	+	-	-	++	++	-	+	-	-

+ = detected; - = absent; CME = Crude methanol extract; NHF = *n*-Hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; NBF = *n*-Butanol fraction; AMF = Aqueous-methanol fraction.

Antioxidant activity

The total phenolic and flavonoid contents, as well as antioxidant activity of both the leaf and stem fractions, are shown in Table 3. The leaf fractions presented higher total phenolic and TFCs than the stem; however, the EAF of both the leaf (447.41 ± 18.77 mgGAE/g) and stem (228.20 ± 0.17 mgGAE/g) depicted higher TPCs than the other fractions. The DCMF and NHF of both the plant parts had the highest TFCs. Regarding the DPPH radical scavenging activity, the NBF followed by the CME of both the leaf and stem showed the best activity, whereas for the ferric reducing antioxidant power, the EAF of both the leaf and stem reflected the optimum result. Among all the tested fractions, there was a strong correlation between the TPC and the ferric reducing antioxidant power ($R = 0.84$).

Anti-inflammatory effect

The anti-inflammatory potential of RD leaf and stem was evaluated by adopting the anti-15 LOX model of inhibition. In general, the leaf extract and its corresponding fractions gave a better inhibition of the enzyme than the stem (Table 4). The EAF of the leaf gave a very promising result presenting an IC_{50} value of 34.14 ± 16.69 μ g/ml, which was better than the reference standard, quercetin, with an IC_{50} value of 54.75 ± 17.07 μ g/ml. The DCMF and NHF of both the parts gave the least activity with an IC_{50} value of 676.11 ± 24.60 and 589.57 ± 103.83 μ g/ml (leaf) and $1,298.58 \pm 409.86$ and 701.52 ± 66.28 μ g/ml (stem), respectively. The AMF of the stem gave the best activity with an IC_{50} value of 96.41 ± 29.75 μ g/ml (Table 4).

Anticholinesterase activity

The CME of both the leaf (19.61 ± 10.36 μ g/ml) and the stem (99.95 ± 3.67 μ g/ml) of RD showed a significant AChE inhibition when individually compared to their respective fractions. The CME (19.61 ± 10.36 μ g/ml) and the AMF ($26.08 \pm$

1.70 μ g/ml) of RD leaf had a significant and optimum inhibitory activity than the standard, eserine (31.23 ± 7.33 μ g/ml), as shown in Table 4.

Antidiabetic activity

α -Amylase inhibitory assay

In this study, the potential of RD leaf and stem and fractions to inhibit α -AL enzyme was compared with that of acarbose, the positive control. For the leaf, the EAF gave the best inhibitory activity of the enzyme with the lowest IC_{50} value of 358.40 ± 3.43 μ g/ml, followed by the NBF (367.50 ± 8.34 μ g/ml) when compared with other fractions and crude extract. There was no significant difference ($p < 0.05$) between the activity of the EAF and NBF, which were close to that of acarbose (342.00 ± 6.30 μ g/ml). CME of the leaf gave the lowest inhibitory activity with an IC_{50} value of 630.30 ± 4.31 μ g/ml (Table 4). However, for the stem, NHF gave the best activity (356.90 ± 4.24 μ g/ml) statistically difference ($p < 0.05$) from other fractions and crude (methanol) extract. This was followed by EAF (405.90 ± 10.41 μ g/ml), whereas DCMF revealed the least α -AL inhibitory activity (Table 4). Above all, the leaf gave a better α -AL inhibitory potential than the stem.

α -Glucosidase inhibitory activity

The DCMF of the leaf (204.00 ± 8.12 μ g/ml) exhibited the best α -GSD inhibitory potential statistically different ($p < 0.05$) when compared to other extracts and control. The activities of the NB and CM fractions are not different ($p > 0.05$) from that of acarbose, the positive control (Table 4). The least inhibitory activity was observed with AMF. The DCMF (222.80 ± 3.54 μ g/ml) of the stem presented the best α -GSD inhibitory activity. This is followed by the EAF (363.50 ± 8.48 μ g/ml), whereas the AMF depicted the least inhibitory activity. The DCMF fraction of both the leaf and stem provided the most effective α -GSD

Table 3. Total phenolic content, TFC and radical scavenging effect of crude and different fractions of RDL and RDS.

Extract/fractions	DPPH IC_{50} (μ g/ml)	FRAP (mgTE/g)	TPC (mgGAE/g)	TFC (mgQE/g)
Leaf				
CME	9.66 ± 1.22^b	36.33 ± 0.67^f	39.96 ± 2.28^c	2.89 ± 0.78^f
NHF	154.32 ± 0.99^c	48.98 ± 2.35^e	29.60 ± 0.70^e	127.13 ± 2.17^a
DCMF	104.45 ± 6.87^d	167.53 ± 1.18^d	230.31 ± 11.78^b	57.57 ± 3.36^b
EAF	103.54 ± 1.22^d	261.98 ± 1.66^b	447.41 ± 18.77^a	6.66 ± 0.93^d
NBF	9.14 ± 0.48^b	53.55 ± 1.75^c	173.20 ± 2.81^c	5.62 ± 0.35^d
AMF	155.65 ± 4.27^c	28.99 ± 1.72^e	35.13 ± 1.05^f	0.94 ± 0.09^e
Stem				
CME	35.24 ± 6.99^c	50.41 ± 2.71^c	39.78 ± 1.84^c	3.15 ± 0.26^f
NHF	409.43 ± 12.14^b	47.69 ± 0.30^e	43.90 ± 8.69^c	13.89 ± 0.82^c
DCMF	190.07 ± 6.65^f	171.41 ± 2.35^c	106.89 ± 3.64^d	14.32 ± 0.26^c
EAF	278.73 ± 0.77^e	281.92 ± 6.23^a	228.20 ± 0.17^b	3.37 ± 0.11^f
NBF	4.40 ± 0.57^a	2.47 ± 0.02^b	27.50 ± 0.10^b	4.80 ± 0.04^e
AMF	509.31 ± 6.70^f	29.78 ± 0.99^e	35.39 ± 0.79^f	2.29 ± 0.46^f

DPPH = 2,2-diphenyl-1-picryl hydrazyl; FRAP = Ferric reducing antioxidant power; TPC = Total phenolic content; TFC = Total flavonoid content.

Values are expressed as mean \pm SEM ($n = 3$). Means with the same letter are not significantly different at $p < 0.05$. IC_{50} of the standards gallic acid (1.72 ± 0.01) and ascorbic acid (3.68 ± 0.09) for DPPH assay

Table 4. Inhibition of 15-lipoxygenase, AChE, α -amylase, and α -GSD enzymes by crude extracts and fractions of RD leaf and stem.

Extract/fractions	15-LOX	AChE	α -amylase IC ₅₀ (μ g/ml)	α -GSDs
Leaf				
CME	74.23 \pm 15.63 ^b	19.61 \pm 10.36 ^a	630.30 \pm 4.31 ^h	275.70 \pm 6.75 ^d
NHF	589.57 \pm 103.83 ^f	227.40 \pm 18.50 ^e	594.20 \pm 7.13 ^g	237.50 \pm 4.72 ^c
DCMF	676.11 \pm 24.60 ^{f,g}	390.90 \pm 14.28 ^b	500.40 \pm 2.87 ^e	204.00 \pm 8.12 ^a
EAF	34.14 \pm 16.69 ^a	409.10 \pm 10.87 ^b	358.40 \pm 3.43 ^b	308.10 \pm 14.99 ^c
NBF	266.63 \pm 69.58 ^c	128.70 \pm 15.85 ^d	367.50 \pm 8.34 ^b	268.80 \pm 9.89 ^d
AMF	85.56 \pm 19.50 ^{b,c}	26.08 \pm 1.70 ^a	397.30 \pm 4.26 ^e	338.50 \pm 7.24 ^f
Stem				
CME	837.84 \pm 66.93 ^g	99.95 \pm 3.67 ^c	460.60 \pm 1.53 ^d	400.20 \pm 7.06 ^b
NHF	701.52 \pm 66.28 ^{f,g}	447.50 \pm 3.04 ^d	356.90 \pm 4.24 ^b	500.30 \pm 1.77 ⁱ
DCMF	1298.58 \pm 409.86 ^{g,h}	458.50 \pm 31.41 ⁱ	815.90 \pm 27.20 ^j	222.80 \pm 3.54 ^b
EAF	175.05 \pm 12.40 ^d	302.30 \pm 14.55 ^e	405.90 \pm 10.41 ^c	363.50 \pm 8.48 ^e
NBF	130.56 \pm 26.35 ^c	149.90 \pm 16.31 ^d	557.90 \pm 7.39 ^f	665.40 \pm 0.50 ^j
AMF	96.42 \pm 29.75 ^{b,c}	248.70 \pm 0.41 ^e	419.70 \pm 4.29 ^e	1337.00 \pm 123.90 ^k
Quercetin	54.75 \pm 17.07 ^{a,b}	–	–	–
Indomethacin	229.19 \pm 33.24 ^e	–	–	–
Eserine	–	31.23 \pm 7.33 ^b	–	–
Acarbose	–	–	342.00 \pm 6.30 ^a	265.20 \pm 6.00 ^d

LOX = lipoxygenase; AChE = acetylcholinesterase.

Values are expressed as mean \pm SEM ($n = 3$). Means with the same letter are not significantly different at $p < 0.05$.

inhibitory activity than other fractions, whereas the AMF gave the least activity for both leaf and stem. In general, the leaf fractions gave a better α -GSD inhibitory activity than the stem.

Mode of inhibition of α -AL and α -GSD enzymes

The DCMF of the leaf with the best α -GSD inhibitory activity and moderate α -AL inhibitory activity was chosen for studying the kinetics of enzymes' inhibition, whereas, in the same manner, the EAF of the stem was chosen for the assay. The modes of α -AL and α -GSD inhibition by DCM (leaf) and EAF of the stem are shown in Figures 1 and 2, respectively. The Lineweaver–Burk plot revealed a non-competitive inhibition of α -AL revealing a reduction in both V_{\max} values among the extract and the control from 0.036 to 0.032 mM/minutes with a constant K_m value (0.35 mM⁻¹) for both control and extract for the leaf (Fig. 1A). Although the mode of α -AL and α -GSD inhibitions for the stem is uncompetitive (Figs. 2A and B), a constant V_{\max} values (0.08 mM/minutes) for both extract and control with a decrease in K_m values from 0.297 (extract) to 0.140 (control) mM/minutes for α -glucosidase (Fig. 1B) suggest a competitive mode of inhibition by the DCMF of the leaf.

DISCUSSION

In this study, the CME of the leaf and stem of RD were first fractionated using different solvents of increasing polarity to obtain different fractions with different phytochemical profiles. Interestingly, the leaf and stem fractions are rich in phenols, alkaloids, and saponins. Subsequently, the antioxidant, anti-inflammatory, anticholinesterase, and antidiabetic properties of RD leaf and stem fractions were investigated to support the ethnomedicinal claims on the use of the plant in traditional

medicine toward the management of neurodegenerative diseases, body inflammation, and diabetes.

EAF was found to contain the highest phenolics, EAF and DCMF (though also high in flavonoids) presented the highest antioxidant activity in the FRAP, and in the DPPH assay, NBF fraction was the best in scavenging the radical followed by the crude extracts of both the plant parts (leaf and stem) going by their lowest IC₅₀ values. Phenolic compounds are reported to have wide pharmacological activities, ranging from their ability to scavenge free radicals (antioxidants) to antidiabetic and anti-inflammatory potentials (Dani *et al.*, 2010). The previous reports had established a relationship between reactive oxygen species and many other diseases such as cancer, diabetes, and neurodegenerative disorder (NDD), particularly AD. In fact, during aging, the antioxidant defense mechanism is compromised or weakened, due to the elevated oxidative stress arising from increased free radical production (Mathew and Subramanian, 2014). The crude extract and AMF of the leaf reflected the most effective AChE inhibition with the IC₅₀ values of 19.61 \pm 10.36 and 26.08 \pm 1.70 μ g/ml, respectively, which is better than that of the standard, eserine (31.23 \pm 7.33 μ g/ml). This result supports the ethnomedicinal claim on the use of *R. dentata* (RD) in the management of the (NDD) (Sonibare and Ayoola, 2015). Hence, RD leaf could be a good therapeutic alternative against NDD, particularly AD. The anticholinesterase effect of the plant could be attributed to the presence of detected alkaloids, known to have cholinesterase inhibitory properties. Current drugs used for the management of AD such as rivastigmine are alkaloids (Mukherjee *et al.*, 2007).

The anti-inflammatory properties of RD leaf and stem fractions were assessed based on the inhibition of 15-lipoxygenase enzyme. The enzyme is important in the bioformation of

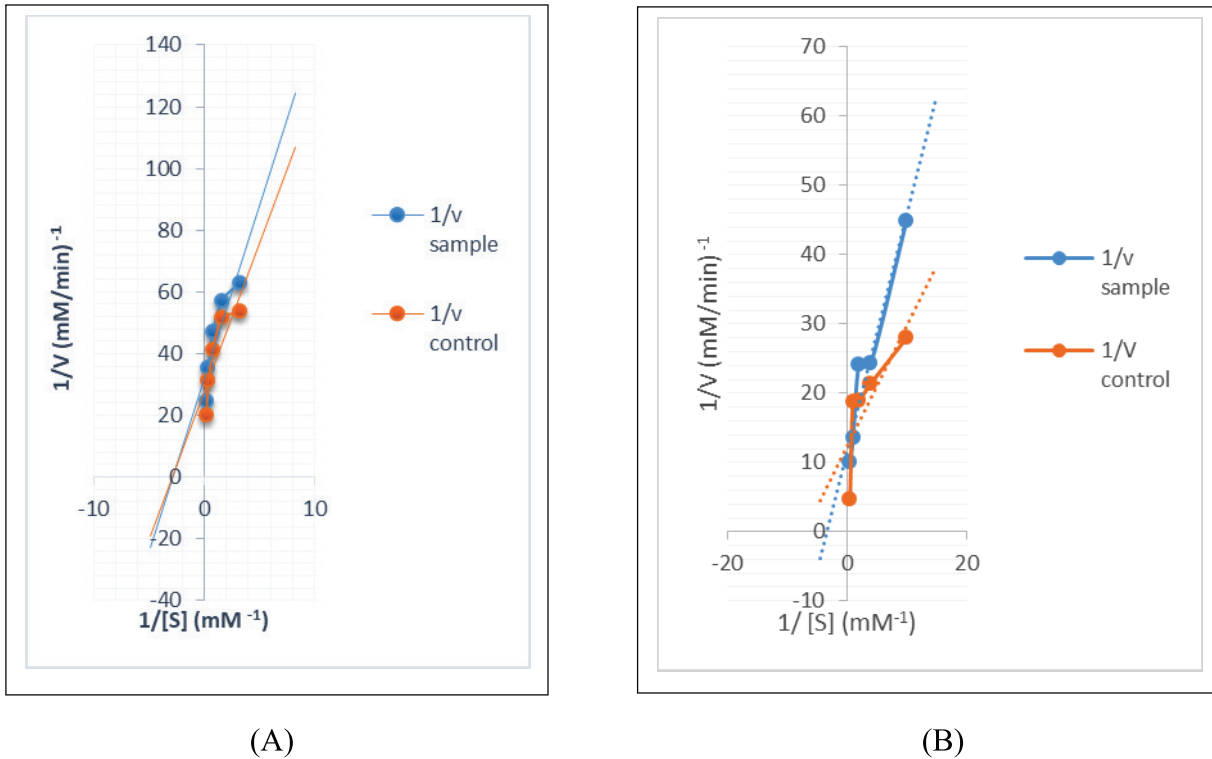


Figure 1. (A) Non-competitive mode of inhibition of α -amylase by DCM fraction of RDL. (B) Competitive mode of the inhibition of α -GSD by DCM fraction of RDL.

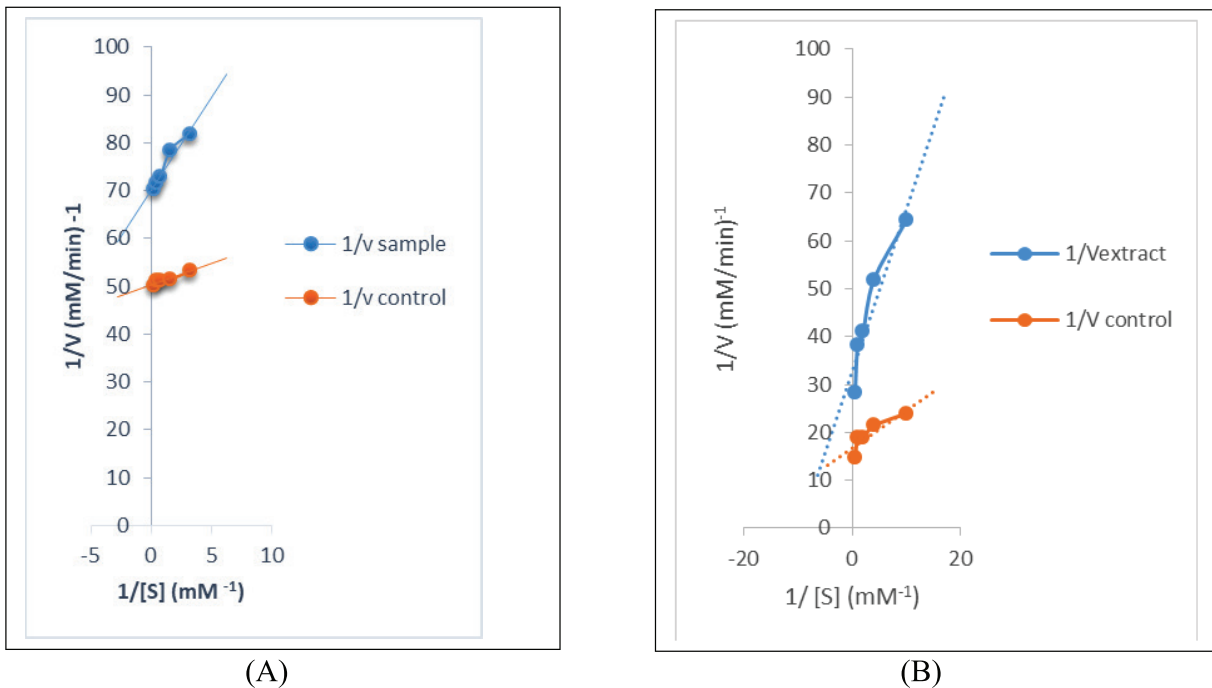


Figure 2. (A) Uncompetitive mode of the inhibition of α -amylase by EA fraction of RDS. (B) Uncompetitive mode of the inhibition of α -GSD by EA fraction of RDS.

leukotriene while triggering the first process in the breakdown of arachidonic acid to leukotrienes (biologically active). Leukotrienes are known as an effective intermediary of inflammatory and allergic responses. The 15-lipoxygenase pathway inhibited with

respect to their pro-inflammatory properties is assumed as the hallmark therapy in the management of numerous inflammation-related diseases (Schneider and Bucar, 2005). The EAF of the leaf gave the best inhibitory activity ($34.14 \pm 16.69 \mu\text{g/ml}$) better

than the positive control, quercetin ($54.75 \pm 17.07 \mu\text{g/ml}$). It is worthy of mention that EAF is rich in phenols as shown by the phytochemical screening result (Table 2) and TPC. The presence of phenolic compounds could be suggested to be responsible for its anti-inflammatory property. Muthuraman *et al.* (2011) and Adebayo *et al.* (2015) attributed a good anti-inflammatory power to the presence or detection of rich amount of phenols in plant extracts. Hence, this fraction could harbor the bioactive compounds that could pave a way for new anti-inflammatory drug discovery. However, indomethacin, which was used as a control in this experiment, had an insignificant or weak lipoxygenase (LOX) inhibitory activity with an IC_{50} value of $229.19 \pm 33.24 \mu\text{g/ml}$. This is as expected because it is a non-selective cyclooxygenase 1 and 2 inhibitor and a non-steroidal anti-inflammatory agent (Hart and Boardman, 1963). This type of poor activity by indomethacin witnessed in this study was also in consonance with the report of Dzoyem and Eloff (2015).

Alpha-amylase and α -GSD enzymes are prominent enzymes involved in the persistent hydrolysis of CHOs to glucose (Mayur *et al.*, 2010). The peak treatment in the management of blood glucose during postprandial hyperglycemia for diabetic patients is the inhibition of the two enzymes. The inhibition of α -AL and α -GSD enzymes was used in this study to evaluate the antidiabetic potential of RD leaf and stem crude extracts and fractions. Besides, a good antihyperglycemic substance that could serve as an alternative treatment for synthetic drugs such as acarbose known with side effects, such as gastrointestinal disturbances, should be mild α -AL and strong α -GSD inhibitors. In line with the aforementioned, DCMF (leaf) showed a mild inhibition of α -AL depicting $500.40 \pm 2.87 \mu\text{g/ml}$ going by its half-maximal IC_{50} value and strong inhibition of α -GSD ($204.00 \pm 8.12 \mu\text{g/ml}$) better than acarbose (IC_{50} : $265.20 \pm 6.00 \mu\text{g/ml}$). Similarly, EAF (stem) showed a mild inhibition of α -AL ($405.90 \pm 10.41 \mu\text{g/ml}$) and strong inhibition of α -GSD ($363.50 \pm 8.48 \mu\text{g/ml}$). Hence, these fractions reflecting good antidiabetic potentials of the plants were studied for the kinetics of inhibition of the two enzymes.

The kinetics of inhibition of the enzymes depicted by the double-reciprocal plot indicated uncompetitive inhibitions of α -AL and α -GSD enzymes, which is evident from the reduction in V_{max} and K_m values for the stem. This suggests that the extract bioactives bind to other position close to the enzyme active site forming complexes with available enzyme (FE) or enzyme substrate (ES), thus having an effect on the activity of both FE and ES (Balogun and Ashafa, 2017) and thereby retarding the activities of these enzymes in breaking down CHOs to glucose. Moreover, the non-competitive inhibition of α -AL for the leaf signifies the binding at other sites instead of the active site of the enzymes. However, the competitive α -GSD inhibition of the leaf indicates that the active components of the leaf extract bind at the active site of the enzyme, thus preventing the binding of the substrate, which is indicative of the fact that the extract has the potential at slowing the constant hydrolysis of disaccharides to glucose. Above all, the reports from this study revealed the presence of secondary metabolites such as saponins, phenols, and alkaloids, which could be responsible for the lowering effect of these extracts on α -AL and α -GSD due to their interaction singly or in combination. Kwon *et al.* (2007) reported that plants' secondary metabolites are

effective inhibitors of α -GSD. Furthermore, Kumar *et al.* (2011) also reported the inhibition of α -GSD by alkaloids and phenols from medicinal plants while the antihyperglycemic potential of alkaloids and saponins from plants are well documented (El-Barky *et al.*, 2017; Elekofehinti, 2015; Yin *et al.*, 2014).

CONCLUSION

In summary, this study revealed the presence of phytochemicals from the leaf and stem of *R. dentata*, the antioxidant property, the anti-inflammatory as well as their anticholinesterase and anti-diabetic potentials. Further works are ongoing and centered toward isolating and identifying important anti-inflammatory, anticholinesterase, and antidiabetic compounds from the leaf of *R. dentata*.

AUTHORS' CONTRIBUTIONS

I.O. did most of the experiments and wrote the manuscript draft. M.S. supervised antioxidant and anticholinesterase experiments. F.B supervised the antidiabetic experiments and manuscript draft. S.A. performed the anti-inflammatory. A.O.T supervised the antidiabetic and anti-inflammatory experiments. All the authors revised the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the Association of African Universities for the small grant for thesis and dissertation given to Ibukun Oresanya and the African-German Network of Excellence in Science for granting a Mobility Grant (2017) to Ibukun Ayoola sponsored by German Federal Ministry of Education and Research as well as the Alexander von Humboldt Foundation. The authors also acknowledge the Directorate Research and Development of the University of the Free State for the postdoctoral research fellowship granted Drs. FO Balogun and S Adebayo tenable at phytomedicine and phytopharmacology research group, Department of Plant Sciences.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None.

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How to cite this article:

Oresanya IO, Sonibare MA, Adebayo S, Balogun FO, Ashafa AOT. Oxidative stress mitigation and the inhibition of carbohydrate-hydrolysing, lipoxygenase (15-LOX), and acetylcholinesterase enzymes by methanolic crude extracts and partitioned fractions of *Rinorea dentata* (P Beauv.) O Ktze. leaf and stem. *J Appl Pharm Sci* 2020; 10(07):064–072.