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# ANTIOXIDANT, ANTI-AMYLASE AND LIPID LOWERING POTENTIAL OF LEAVES OF *Aporosa lindleyana* BAILL. (KEBELLA)

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## Abstract

Globally, the demand for herbal products with antioxidant capacity is increased due to many health benefits. The approach to discover new drugs through natural products has become a successful strategy in recent years. *Aporosa lindleyana* Baill.(Kebella) is used as a leafy vegetable in Sri Lanka. Although the root and bark of this plant showed many biological activities, only antioxidant activity by DPPH mechanism and anti-inflammatory activity are reported in leaf. The present study investigated the antioxidants [Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)], antioxidant activities [Ferric Reducing Antioxidant Power (FRAP),1,1-diphenyl-2-picrylhydrazyl radicalscavenging assay (DPPH),2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical scavenging assay (ABTS) and Oxygen Radical Absorbance Capacity (ORAC)], anti-amylase activity(using acarbosestandard)and anti-lipidaemicactivities via anti-lipase (AL), anti-cholesterolesterase (AC) and bile acids binding (BAB) *invitro* of95% ethanolic extract (EE) and water extract(WE)of *A.lindleyana*leaves along with its nutritional value.Both extracts showed significant differences ($P<0.05$) among the investigated antioxidants and antioxidant activities. For all the studied antioxidant activities, the EE showed high activity compared to WE and lower antioxidants in TPC. EE showed higher anti-amylase activity ($IC_{50}$:164.85±8.36 µg/mL) compared to WE ($IC_{50}$: 746.25±53.64 µg/mL). The $IC_{50}$ of the standard drug, a carbose is 133.88 ± 4.4 µg/mL. Both extracts had dose dependant anti-lipidaemic activities in terms of anti-lipase, anti-cholesterol esterase and bile acids binding. However, EE showed significantly higher($p<0.05$)anti-lipase and anti-cholesterol esterase activities compared to WE. In contrast, WE showed significantly higher ($p<0.05$) BAB for studied bile acids compared to EE (excepttaurocholate binding). Both extracts showed high taurocholate binding,similar binding of glycodeoxycholate and chenodeoxycholate and moderate anti-lipase activity compared to the reference drug (orlistat) studied.The proximate composition: moisture, carbohydrate, protein, crude fat, crude fibre and ash were 12.2, 49.4, 13.2, 1.1, 16.0 and 8.1%respectively. The minerals (Na, Mn, Fe, P, Zn, Cu: 53.7, 49.3, 41.4, 27.6, 22.6 and 10.4mg/kg; K, Ca and Mg: 18, 3.2and 2.7 g/kg, respectively) were also present. The leavesof *A. lindleyana* possess marked antioxidant, anti-amylase and moderate lipid lowering activities. This is the first report on multiple mechanisms of antioxidant activities, anti-amylase and lipid lowering effects which indicates the potential of developing *A. lindleyana* leaves as a functional food in prevention and management of non-communicable diseases.

Key words: *Aporosa lindleyana*, antioxidant, anti-amylase, lipid lowering activity, nutritional value

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## 1. Introduction

Globally, the demand for herbal products with antioxidant capacity is increased due to many health benefits. Plants have been major contributors to human life since the dawn of civilization. The approach to discover new drugs through natural products has become a successful strategy in recent years (Soumya *et al.*, 2009; Ediriweera and Ratnasooriya 2009).

*Aporosalind leyana* Baill. (Family: Euphorbiaceae; Tamil: Vittil; Sinhala: Kebella) is used as a leafy vegetable in Sri Lanka. The root of this plant is used as a treatment for insanity, head ache, fever, diabetes, loss of semen and liver diseases. The sweet and sour seeds with fleshy arils, separated from mature fruits are consumed (Aswathi *et al.,* 2017). Kalashaka Yoga, an ayurvedic pill (125 mg, two pills twice a day) containing the dried plant materials (bulb of *Allium sativum*, leaves of *Murrayakoenigii,* seeds of *Piper nigrum*, leaves of *Aporosalindleyana*andleaves of *Olaxzeylanica* in 4: 4: 1: 2: 2 ratio) mixed with bees honey is proven to be effective in hyper cholesterolemicpatients (Ediriweera *et al.,* 2007). Although the root andbark of this plant are reported to have many health benefits, there is limited scientific data on the activity of leaves(Srikrishna *et al.,* 2008) except the antioxidant activity by DPPH mechanism (Samanmali *et al.,* 2014) and anti-inflammatory activity (Aswathi *et al.,* 2017). The present study therefore, investigated the antioxidant content by Total Phenolic content (TPC) and Total Flavonoid Content (TFC)and antioxidant activities by multiple mechanisms: FRAP, DPPH, ABTS and ORAC assays, anti-amylase activity (using acarbose standard) and anti-lipidaemicactivities via anti-lipase (AL), anti-cholesterol esterase (AC) and bile acids binding (BAB) *in vitro* for 95% ethanolic extract (EE)and water extract (WE)of *A. lindleyana* leaves.

## 2. Material and Methods

### *2.1 Chemicals and equipment*

Folin-Ciocalteu reagent, gallic acid, quercetin, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid(Trolox),1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), potassium persulphate,2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, 2,4,6-tripyridyl-s-triazine (TPTZ), 4,4'-disulfonic acid sodium salt (ferrozine),3,5- dinitrosalicylic acid (DNS), acarbose, porcine pancreatic lipase (PPL, type II), 4-nitrophenyl butyrate (p-NPB), cholesterol esterase from porcine pancreas, sodium taurocholate hydrate, sodium chenodeoxycholate, sodium glycodeoxycholate, orlistat and cholestyramine resin were purchased from Sigma-Aldrich Co. St. Louis, MO, USA. *α*-Amylase *(Bacillus amyloliquefaciens)* was purchased from Roche Diagnostics, USA and total bile acid kits (BQ 042A-EALD) were purchased from Bio-Quant Co. (San Diego, CA, USA). All other chemicals used for the preparation of buffers and solvents were of analytical grade. All the analyses were carried out using High-throughput 96-well micro-plate readers (SpectraMax Plus384, Molecular Devices, USA and SPECTRAmax-Gemini EM, Molecular Devices Inc, USA).

*2.2 Plant material*

Fresh leaves of *A. lindleyana* were collected from Maharagama, Sri Lanka during April to October 2015. The specimen of the plant was identified and authenticated in Herbal Technology Section, Industrial Technology Institute and the herbarium specimen was deposited.

***2.3** Preparation of WEand EE of Aporosa lindleyana leaves*

Fresh leaves were cleaned, oven dried at 50 °C for 6 h and powdered. Powdered leaves, 20 g each were extracted with water and 95% ethanol for 6 h separately using refluxing method and Soxhlet extraction, respectively (Wang and Weller, 2006). These extracts were dissolved in DMSO (<2%) and used to evaluate the antioxidant properties, anti-amylase activity and lipid lowering effects.

*2.4 Antioxidant activity*

Antioxidants (TPC and TFC) and antioxidant activities (FRAP,DPPH,ABTS and ORAC) of EE and WE of *A. lindleyana* leaves were studied according to the methods described by Singleton*et al.,*1999 for TPC; Siddhuraju P and Becker K., 2003 for TFC;Benzine IFF and Szeto YT., 1999; Blois MS., 1958for DPPH; Re *et al.,* 1999 for ABTS and Ou B *et al.,* 2001 for ORAC with some modifications using 96-well micro plates, *in vitro* (TPC: EE, n=3 and WE, n=6; TFC: EE and WE, n=3 each; FRAP: EE and WE,n=3 each; ORAC: EE,n=8 and WE, n=4; DPPH and ABTS: EE and WE, n=4 each).

*2.5 Anti-amylase activity*

Anti-amylase activity of WE and EE were studied according to the methods described by Bernfeld, 1955 with some modifications. A reaction volume of 1 mL containing 50 µL of WEand EE at concentrations 93.75, 187.50, 375, 750, 1500 µg/mL (n=4 each), 40 µL of starch (1%, w/v) and 50 µL of enzyme,α-amylase (5 µg/mL) in 100 mM sodium acetate buffer (pH 6.0) were incubated at 40 °C for 15 min. After the incubation period, 0.5 mL of DNS reagent was added and placed in a boiling water bath for 5 min and allowed the reaction mixtures to cool in a water bath containing ice for 15 min. The absorbance readings were recorded at 540 nm using a 96-well micro plate reader (SPECTRAmaxPLUS384 Molecular Devices, Inc, USA). Control experiment contained all the reagents except leaf extracts whereas, sample blanks were without the enzyme. Acarbose was used as the positive control (6.25 – 100 µg/mL). Anti-amylase activity (% inhibition) was given as $IC_{50}$ values (Concentration of WE and EE and positive control that inhibited the hydrolysis of starch by 50%). Inhibition % was calculated using the following equation.

$$\text{Inhibition (\%)} = [A_c - (A_s - A_b)/Ac]*100$$

Where, $A_c$ is the absorbance of the control, $A_b$is the absorbance of sample blanks and $A_s$ is the absorbance in the presence of extracts.

*2.6 Lipase inhibition assay*

Pancreatic lipase inhibitory activity of WE and EEwas carried out according to the method described by Kim *et al.,* 2010 with some modifications. Reaction volume of 200 µL, containing 30 µL of 2.5 mg/mL porcine pancreatic lipase (PPL, type II) enzyme and 120 µL of different concentrations of WEand EE (assay concentrations: 1000, 500, 250, 125, 62.5 µg/mL) in 0.1 M TrisHCl buffer with 5 mM $CaCl_2$, pH 7.0 were pre-incubated at 37 °C for 15 min. Reaction was started by adding 5 µL of 10 mM, p-NPB in dimethylformamide and was allowed to proceed at 37 °C for 30 min. Lipase inhibitory activity of both extracts were determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using a micro plate reader. Inhibition of lipase activity was expressed as the

percentage decrease in optical density when pancreatic lipase was incubated with both extracts. Lipase inhibition (%) was calculated according to the following formula and anti-lipase activity is given as $IC_{50}$ values (the concentrations of both extracts and the positive control that inhibited the hydrolysis of p-NPB to p-nitrophenolby 50%, n=3). Orlistat was used as the positive control (assay concentration: 0.20 – 6.25 µg/mL, n=3).

The percentage inhibition was calculated as:

$$\text{Inhibition } (\%) = [(A - a) - (B - b)]100/(A - a)$$

Where, A is the activity without inhibitor, a- the negative control without inhibitor, B- the activity with inhibitor and b- the negative control with inhibitor.

*2.7 Cholesterol esterase inhibition assay*

Pancreatic cholesterol esterase inhibitory activity of WEand EE of leaves was performed according to the method reported by Pietsch and Gütschow, 2005 with minor modifications. Reaction volume of 200 µL, containing different concentrations of both extracts (assay concentrations: 600, 300, 150, 75, 37.5, 18.75 µg/mL) were pre-incubated with 50 µL of 24 mMtaurocholic acid, 5 µL of 8 mM p-NPB in acetonitrile in 0.1 M sodium phosphate buffer, 0.1 M NaCl, pH 7.0 at 25 °C for 10 min. Reaction was started by adding 42.5 µL of (1.25 µg/mL) cholesterol esterase enzyme and change in absorbance was monitored at 405 nm at 25 °C for 6 min using SpectraMax384 micro plate reader. The kinetic parameter Vmaxwas used to calculate the % inhibition and cholesterol esterase inhibitory activityisgiven as $IC_{50}$ values (the concentrations of both extracts that inhibited the hydrolysis of p-NPB to p-nitrophenol by 50%, n=4).

The percentage inhibition was calculated as:

$$\text{Inhibition } (\%) = [(A_C - A_S)/A_C]*100$$

Where $A_C$ is the Vmaxof the control and $A_S$ is the Vmaxof the sample.

*2.8 Bile acid binding assay*

The effect of *A. lindleyana*WEand EE of leaves on bile acid binding wasperformed according to the method reported by Adisakwattana*et al.,* 2012 with some modifications. Taurocholic acid, glycodeoxycholic acid and chenodeoxycholic acid were used as the bile acids. Leaf extracts (assay concentrations: 3, 2 and 1 mg/mL; n=4) were incubated with each bile acid (2 mM) in 0.1 M phosphate buffer (PBS), pH=7, at 37 °C for 90 min. Each bile acid without extract was used as the control. The mixtures were filtered through 0.22 µm filter to separate the bound bile acids from the free bile acids and were frozen at –20 °C until the analysis was carried out. The bile acid concentration was analyzed spectrophotometrically at 540 nm by using bile acid analysis kit (BQ 042A-EALD). Cholestyramine resin was used as the positive control (assay concentration: 3, 2 and 1 mg/mL, n=4).

*2.9 Proximate analysis*

The quantification of proximate composition (moisture: AOAC 2012 - 930.04; crude protein: AOAC 2012 - 2001.11; crude fibre: AOAC 2012 - 920.09; ash: AOAC 2012 - 942.05) of kebella leaf powder was carried out.

*2.10 Statistical analysis*

Data represented as mean ± SE. Data of each experiment were statistically analyzed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences among treatment means. $P<0.05$ was regarded as significant.

## 3. Results

*3.1* Antioxidant activity

Summary of results of antioxidant properties of WE and EEof *A. lindleyana* leaves of are given in Table 1.

Dose response relationship of EE and WE for DPPH and ABTS radical scavenging activities are given in Table 2 and 3, respectively.

**Table 1.**Antioxidant properties of WE and EEof *A. lindleyana* leaves

| Extract | Antioxidant | | Antioxidant Activity | | | |
|---|---|---|---|---|---|---|
| | TPC (mg gallic acid equivalents/ g of extract) | TFC (mg quercetin equivalents/ g of extract) | FRAP (mg Trolox equivalents/ g of extract) | ORAC (mg Trolox equivalents/ g of extract) | DPPH (mg Trolox equivalents/ g of extract) | ABTS (mg Trolox equivalents/ g of extract) |
| EE | 264.35±3.84$^{b}$ | 6.86±0.12$^{a}$ | 369.87±6.88$^{a}$ | 321.49±5.95$^{a}$ | 337.61±10.15$^{a}$ | 506.48±5.27$^{a}$ |
| WE | 297.91±2.75$^{a}$ | 0.94±0.20$^{b}$ | 329.94±3.55$^{b}$ | 304.79±5.27$^{b}$ | 277.73±2.09$^{b}$ | 470.55±4.93$^{b}$ |

Data represented as mean±SE. TPC: EE n=3 and WE, n=6; TFC: EE and WE, n=3 each; FRAP: EE and WE,n=3 each; ORAC: EE, n=8 and WE, n=4; DPPH and ABTS: EE and WE, n=4 each). Values in the column superscripted by different letters within a column are significantly different ($p<0.05$).

**Table 2.**Dose response relationship of WE and EE of *A. lindleyana* leaves for DPPH radical scavenging activity

| Concentration (µg/mL) | % Radical scavenging | |
|---|---|---|
| | EE | WE |
| 62.5 | 85.56±0.40 | 80.64±1.32 |
| 31.25 | 71.08±2.79 | 58.22±0.59 |
| 15.625 | 36.99±0.74 | 30.23±0.41 |
| 7.8125 | 17.55±0.84 | 10.54±0.27 |
| 3.90625 | 8.02±0.55 | 1.71±0.25 |
| $IC_{50}$ | 1.94±0.64$^{b}$ | 26.63±0.20$^{a}$ |

Data represented as mean ± SE; EE and WE n=4 each and $r^2$ 0.99 & 0.99 respectively; $IC_{50}$ Trolox = 7.40±0.32 µg/mL.Values in the columns superscripted by different letters are significantly different at $p<0.05$. $IC_{50}$ = Concentration of EE and WE at 50% inhibition of DPPH radical.

**Table 3.**Dose response relationship of WE and EE of *A. lindleyana* leaves for ABTS radical scavenging activity

| Concentration (µg/mL) | % Radical scavenging | |
|---|---|---|
| | EE | WE |
| 20 | 71.64±0.95 | 74.92±0.50 |
| 15 | 70.53±0.58 | 51.64±2.07 |
| 10 | 29.10±0.38 | 30.87±0.49 |
| 7.5 | 30.70±1.88 | 34.49±0.89 |
| 5 | 7.03±0.14 | 9.02±0.35 |
| $IC_{50}$ | **13.46±1.78[a]** | **14.46±0.15[a]** |

Data represented as mean ± SE; EE and WE, n=4 each and $r^2$ 0.91 & 0.96 respectively; $IC_{50}$ Trolox = 6.80±0.41 µg/mL.Values in column superscripted by same letter are significantly not different at $p<0.05$. $IC_{50}$ = Concentration of ethanol and water extracts at 50% inhibition of ABTS radical.

*3.2 Anti-amylase activity*

Anti-amylase activity of EEand WE of *A. lindleyana* leaves is given in Table 4.

*3.3 Lipid lowering effect*

The inhibitory activity of lipase and cholesterol esterase and the binding of taurocholate, glycocholate and chenodeoxycholate bile acids of WE and EEof leaves of *A. lindleyana* are given in Table 5, 6 and 7 respectively.

*3.4 Proximate composition*

The proximate composition: moisture, carbohydrate, protein, crude fat, crude fibre and ash are 12.2, 49.4, 13.2, 1.1, 16.0 and 8.1%, respectively. The presence of crude fibre in the leaves of *A. lindleyana* is helpful to those who have constipation.

*3.5 Mineral content*

The leaves of *A. lindleyana* are rich in minerals. The mineral content is reported in Table 8.

**Table 4**. Anti-amylase activity of WE and EEof *A. lindleyana* leaves

| Concentration (µg/mL) | % Inhibition | |
|---|---|---|
| | EE | WE |
| 1500 | 99.80±12.12 | 58.59±6.30 |
| 750 | 88.10±8.75 | 65.26±4.60 |
| 375 | 68.38±3.81 | 40.50±2.98 |
| 187.5 | 53.64±1.52 | 25.53±1.87 |
| 93.75 | 36.36±3.86 | 17.20±5.24 |
| $IC_{50}$ | **164.85±8.36[a]** | **746.25±53.64[b]** |

Data represented as mean ± SE; EE and WE and acarbose, n=4each; EE and WE, $r^2$: 0.99 and 0.96 respectively; $IC_{50}$ of acarbose: 133.88±4.4 µg/mL; Acarbose,$r^2$: 0.99. Values in the columns superscripted by different letters are significantly different at $p<0.05$.

**Table 5.** Anti-lipase activity of WE and EEof leaves of *A. lindleyana*

| Concentration (µg/mL) | % Inhibition | |
|---|---|---|
| | EE | WE |
| 1000 | 21.41±6.77 | 9.09±1.27 |
| 500 | 15.59±6.42 | 11.69±7.62 |
| 250 | 16.14±1.68 | 11.19±1.54 |
| 125 | 6.85±4.74 | 8.94±2.95 |
| 62.5 | 3.17±3.15 | 4.08±5.12 |

. Data were represented as mean±SE; n=4 each. Standard drug: orlistat, $IC_{50:}$ 26.78±2.45 µg/mL

**Table 6.** Anti-cholesterol esterase activity of WE and EEof leaves of *A. lindleyana*

| Concentration (µg/mL) | % Inhibition | |
|---|---|---|
| | EE | WE |
| 600 | 43.13±1.79 | 21.91±12.11 |
| 300 | 40.32±3.13 | 20.85±1.72 |
| 150 | 36.55±3.79 | 18.30±2.40 |
| 75 | 25.96±6.30 | 17.88±1.90 |
| 37.5 | 9.31±6.18 | 14.32±2.63 |
| 18.75 | 7.36±0.63 | 7.76±2.96 |

Data were represented as mean±SE; n=4 each.

**Table 7.** The % binding for taurocholate, glycocholate and chenodeoxycholate bile acids of leaves of *A. lindleyana*

| Bile acid | Extract concentration (mg/mL) | % Binding | | |
|---|---|---|---|---|
| | | EE | WE | Standard drug (cholestyramineresin) |
| Taurocholate | 1 | 12.20±3.20$^a$ | 12.49±0.92$^a$ | 4.43±1.15$^b$ |
| | 2 | 42.19±1.75$^a$ | 36.01±1.01$^a$ | 8.23±0.49$^b$ |
| | 3 | 41.79±4.37$^a$ | 40.95±0.55$^a$ | 11.25±1.27$^b$ |
| Glycocholate | 1 | 27.07±5.47$^b$ | 35.75±2.31$^a$ | 37.02±2.59$^a$ |
| | 2 | 29.02±1.17$^b$ | 69.91±3.23$^a$ | 69.02±1.92$^a$ |
| | 3 | 32.45±2.26$^b$ | 67.57±1.91$^a$ | 71.99±2.28$^a$ |
| Chenodeoxycholate | 1 | 11.90±0.07$^b$ | 27.60±1.49$^a$ | 25.53±3.30$^a$ |
| | 2 | 28.85±1.85$^b$ | 57.53±5.50$^a$ | 50.14±3.04$^a$ |
| | 3 | 31.84±1.65$^b$ | 62.09±10.14$^a$ | 66.51±1.95$^a$ |

Data were represented as mean±SE; n=4 each. Mean % binding values within each row superscripted by different letters are significantly different ($p<0.05$).

**Table 8.** Mineral content of dried *Aporosalindleyana* leaf powder

| Minerals | Amount |
|---|---|
| Sodium (Na) | 53.7mg/kg |
| Manganese (Mn) | 49.3 mg/kg |
| Iron (Fe) | 41.4 mg/kg |
| Phosphorus (P) | 27.6 mg/kg |
| Zinc (Zn) | 22.6 mg/kg |
| Copper (Cu) | 10.4 mg/kg |
| Potassium (K) | 18 g/kg |
| Calcium (Ca) | 3.2 g/kg |
| Magnesium (Mg) | 2.7 g/kg |

## 4. Discussion

### *4.1 Antioxidant activity*

Both WE and EE of leaves of *A. lindleyana* possess antioxidants in TPC and TFC and antioxidant activities in FRAP, DPPH, ABTS and ORAC. However, EE and WE showed significant differences ($P<0.05$) among the investigated antioxidants and antioxidant activities. EE showed high activity for all the studied antioxidant activities compared to WE and lower antioxidants in TPC. The TPC and TFC of EE of leaves of *A. lindleyana* were 264.35±3.84 mggallic acid equivalents (GAE)/g of extract and 6.86±0.12 mg quercetin equivalents (QE)/g of extract, respectively while the antioxidant activities of FRAP, DPPH, ABTS and ORAC were369.87±6.88 mg trolox equivalents (TE)/g of extract, 337.61±10.15 mg TE/g of extract, 506.48±5.27 mg TE/g of extract and 321.49±5.95 mg TE/g of extract respectively. Similarly, WE had 297.91±2.75 mg GAE/g of extract, 0.94±0.20 mg QE/g of extract of TPC and TFC, respectively while FRAP, DPPH, ABTS and ORAC had 329.94±3.55 mg TE/g of extract, 277.73±2.09 mg TE/g of extract, 470.55±4.93 mg TE/g of extract and 304.79±5.27 mg TE/g of extract respectively.

### *4.2 Anti-amylase activity*

EE showed higher anti-amylase activity ($IC_{50}$: 164.85±8.36 µg/mL) compared to WE ($IC_{50}$: 746.25±53.64 µg/mL). The standard drug acarbose had $IC_{50}$: 133.88±4.4 $\mu$g/mL.

### *4.3 Lipid lowering effect*

Bile acids are derived from cholesterol in the liver. The synthesis of bile salts is the major route for elimination of cholesterol from the body. During bile acid biosynthesis from cholesterol, the primary bile acid, chenodeoxycholic acid is formed by an alternative or acidic pathway and glycodeoxycholic and taurodeoxycholic acids by classic pathway. When EE and WE of *A. lindleyana* leaves evaluated for lipid lowering effect, both EE and WE had dose dependant anti-lipidaemic activities in terms of anti-lipase, anti-cholesterol esterase and bile acids binding. However, ethanolic extract showed significantly higher ($p<0.05$) anti-lipase (21.41±6.77% inhibition at 1 mg/mL) and anti-cholesterol ($IC_{50}$:720.07±26.81 µg/mL) activities compared to water extract (AL: 9.09±1.27% inhibition at 1 mg/mL; AC: 21.91±0.58% inhibition at 600 µg/mL). In contrast, WE showed significantly higher ($p<0.05$) BAB for studied bile acids compared to EE (except taurocholate binding). The BAB of WE of *A. lindleyana* for glycodeoxycholate and chenodeoxycholate ranged from 35.75±2.31 -67.57±1.91% and 27.60±1.49 - 62.09±10.14% respectively. Similarly, EE had 27.07±5.47 - 32.45±2.26 and 11.90±0.07 - 31.84±1.65% respectively.Further, both EE and WE showed similar BAB for taurocholate bile acid (% bindingEE: 12.49±0.53 - 40.95±0.55; WE: 12.20±1.85 - 41.79±4.37). Both extracts showed high taurocholate binding, similarglycocholate and chenodeoxycholate binding and moderate anti-lipase activity compared to the reference drugs studied (Eskandar and Somayeh, 2015). The lipid lowering effect on EE and WE of *A. lindleyana* may

be due to the reduction of cholesterol by classic pathway.

*4.4 Proximate composition*

*A. lindleyana* leaves are rich in carbohydrate, protein and crude fibre. The leaves, when consumed as a food, gives energy and can be beneficial for those who have constipation due to their high fibre content.

*4.5 Mineral content*

Only a few leafy vegetables have been reported to have more minerals in sufficient quantity, but the results for the analysis of minerals in *A. lindleyana* leaves showed it contain almost all the minerals in required amount (Table 8).

## 5. Conclusion

The leaves of *A. lindleyana* possess marked antioxidant and anti-amylase activities and moderate lipid lowering effects in terms of anti-lipase, anti-cholesterol esterase and bile acids binding. The leaves contain almost all the minerals in sufficient amounts along with the macro-nutrients, carbohydrate, protein and fat. This is the first report on antioxidant activity by multiple mechanisms, anti-amylase and anti-lipidaemic activities via anti-lipase, anti-cholesterolesterase and bile acids binding of leaves of *A .lindleyana* and indicates its potential for development of functional foods and neutraceuticals in the prevention and management of non-communicable diseases worldwide.

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## Conflicts of Interest

The authors declare no conflict of interest.

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