

# Estimation Of The Preliminary Screening Of Phytochemicals, Total Phenolic Content, Total Flavonoid Content, Antioxidant And Alpha-Amylase Inhibitory Activities Of The Selected South African Plant Extracts For Medicinal Purposes.

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**Abstract-** Medicinal plants serve as important sources of pharmaceutical, cosmetic and traditional medicine. Natural products continue to play a significant role in drug discovery and development. Bioactive compounds, which naturally occurs in medicinal plants, such as antioxidants, play a vital role in scavenging free radicals. The purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha ( $\alpha$ )-amylase potential on selected South African plant extracts.

In qualitative analysis, the phytochemical compounds such as tannins, saponins, flavonoids, quinones, phenols, terpenoids, alkaloids, glycosides, cardio glycosides, coumarins, betacyanin, Anthocyanin, and Steroids were screened and quantified. In addition to this, antioxidants were analysed using DPPH and ABTS radical scavenging assays. Out of thirteen qualitative phytochemicals analyzed, ten phytochemicals were identified. The antioxidant inhibition was observed in four plant species whose scavenging activity was above 80% are *Schubria pinnata*>*Lippia javanica*>*Clerodendrum myricoides*>*Erythrina lysistemon*> respectively. Also, these plant species exhibited an alpha-amylase inhibitory effect of 80%. The IC<sub>50</sub> values were > 1000  $\mu$ g/ml. These findings suggest that the presence of phenolic, flavonoids, antioxidants, and  $\alpha$ -amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases.

**Index Terms-** phytochemicals, phenolic, flavonoid, antioxidants, alpha-amylase, medicinal plants.

## I. INTRODUCTION

Phytochemicals are chemicals produced by plants either through primary or secondary metabolism (Kennedy and Wightman, 2011). In general, phytochemicals play an important role in protecting plant growth or act as a defence against competitors, pathogens, or predators (Saxena *et al.*, 2013). Importantly, phytochemicals have been reported to protect humans against diseases (Roa, 2003). The protective role of secondary metabolites extends to antioxidants free radical-scavenging, UV light-absorbing, antimicrobial, and anti-proliferative agents (Saxena *et al.*, 2013, Wink, 2003, Kurutas, 2016). Antioxidants have also been defined as chemical substances that protect the body cells from injury by free radicals (Lobo *et al.*, 2010). As delineated by (Knight, 1995, Lobo, 2010), free radicals attribute to more than one hundred disorders in humans, including atherosclerosis, arthritis, ischemia-reperfusion injury of many tissues, central nervous system injury, gastritis, and cancer. Furthermore, the protective effects from antioxidants have been observed to be useful towards the prevention of lipids damage, protein, and DNA using preventing radicals initiation, breaking chain propagation or suppressing formation (Kurutas, 2016). Moreover, these antioxidants can prevent deteriorating diseases such as cancer, and also slow down the ageing process (Bahense *et al.*, 2017, Kada *et al.*, 2017). These metabolites are essentially used in traditional medicine to treat and manage certain diseases (Boadu and Asase, 2017).

The phytochemicals that are extracted from plants have been classified into three major groups which are terpenoids, phenolic metabolites, and alkaloids (Harborne *et al.*, 1999). Approximately 10, 000 phytochemicals have been identified thus far (Zhang *et al.*, 2015). The most important of these phytochemicals are alkaloids, tannins,

flavonoids, saponins, glycosides, and other phenolic compounds, due to their biological activities screened from medicinal plants thus far (Dluya *et al.*, 2017, Ali, 2011, Bossou *et al.*, 2013, Mladenka *et al.*, 2018, Sayhan *et al.*, 2017, War *et al.*, 2012).

Essentially, very few of these medicinal plants have been explored for their phytochemicals, and pharmacological potential so the chance of finding plants with medicinal uses exist (Mgbeahuruike *et al.*, 2017, Street and Prinsloo, 2013). Furthermore, bioactive compounds with anti-inflammatory, antioxidant and antidiabetic properties are potential solutions towards the management of diabetes, and other chronic inflammatory diseases (Huang *et al.*, 2016). The free radicals are naturally formed in the body, and play a pivotal role in many normal cellular processes (Diplock *et al.*, 1998, Valko *et al.*, 2007). However, at high concentrations, the free radicals can be hazardous to the body and may damage all major components of cells, including DNA, proteins, and cell membranes (Phaniendra *et al.*, 2015). The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes, and other chronic health conditions (Halliwell, 1994, Diplock *et al.*, 1998, Valko *et al.*, 2007). Africa is host to a large percentage of the global floral diversity (Aslan *et al.*, 2013). Only a few of these plants have been explored for their pharmacological potential meaning most of these plants remain untapped, especially in the southern African region (Street and Prinsloo, 2013). Therefore, the purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha ( $\alpha$ )-amylase potential on selected South African plant extracts.

## 1. Materials and methods

### 1.1 Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade; which were quercetin, gallic acid, ascorbic acid, aluminium chloride, sodium carbonate, sulphuric acid, ferric chloride, Fehling's A and B, Rutin, folin Cocteau reagent, dragendorff reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

### 1.2 Plant collection and extraction

Plant species (n=9) were collected from Walter Sisulu National Botanical Gardens in South Africa; in February 2017 (**Table 1**). The voucher specimen number is held at Walter Sisulu National Botanical Gardens herbarium. The plant material was air-dried in a well-ventilated room. After drying, the plants were grounded into a powder and stored away from light at room temperature.

**Table 1: Accession numbers and voucher specimen numbers of the plant species used in this study.**

NAME	FAMILY	PART	Accession nUMBER	VOUCHER OF SPECIMEN COLLECTED	
				Date	NUMBER
<i>Euclea crispa</i>	Ebenaceae	Leaf	24/1982	11/10/1982	24, Behr, C.M
<i>Euclea natalensis</i>	Ebenaceae	Leaf	178/1987	10/6/1987	479; Steel, B.S
<i>Schkuhria pinnata</i>	Asteraceae	Leaf	N/A	N/A	N/A
<i>Ziziphus mucronata</i>	Rhamnaceae	Leaf	36/1982	15/10/1982	39; Behr, C.M
<i>Ziziphus mucronata</i>	Rhamnaceae	Fruits	36/1982	15/10/1982	39; Behr, C.M
<i>Lippia javanica</i>	Verbenaceae	Leaf	16/2014	22/1/2014	28; Kondlo, M
<i>Vernonia oligocephala</i>	Asteraceae	Leaf	268/2013	12/05/2013	29; Hankey, A.J
<i>Clerodendrum myricoides</i>	Lamiaceae	Leaf	11/1987	2/2/1987	367, Steel, B.S

<i>Erythrina lysistemon</i>	Fabaceae	Leaf	21/1982	7/10/1982	22; Behr, C.M
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### 1.3 Preparation of plant extracts

The grounded plant extracts (leaves, and fruits) were extracted with 90% methanol (1 g/10 ml), and vigorously shaken for 3 h. The crude extracts were filtered through Whatman No.1 filter paper and dried at room temperature under the stream of cold air. The crude extracts were reconstituted in a solvent of choice at a concentration of 10 mg/ml.

### 1.4 Qualitative phytochemical tests

#### 1.4.1 Test for alkaloids

A few drops of concentrated hydrochloric acid (HCl) were added to 2 ml of each plant extract solution in a test tube. Then, the dragendorff reagent was added to the solution. The formation of orange precipitation indicated the presence of alkaloids.

#### 1.4.2 Test for cardio glycosides

Plant extract (0.25 g) was dissolved into 1 ml methanol (MeOH) into a test tube. Two ml of glacial acetic acid and a few drops of 5% ferric chloride were added. In addition to this, 1 ml of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added on the side of the test tube. The formation of the brown ring at interface indicates the presence of cardio glycosides.

#### 1.4.3 Test for flavonoids

Plant extract (0.75 g) was dissolved in 3 ml of MeOH in a test tube. Four ml of 1 N sodium hydroxide (NaOH) was added into the test tube. The formation of dark yellow colour was observed which indicated the presence of flavonoids.

#### 1.4.4 Test for phenols

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. Two ml of distilled water was added into a test tube followed by 0.5 ml of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and 0.5 ml Folin Ciocalteuau's reagent. The formation of a blue/green colour indicates the presence of phenols.

#### 1.4.5 Test for saponins

Plant extract (0.5 g) was dissolved into 2 ml of boiling water in a test tube. This was allowed to cool, and shaken well to mix thoroughly. The presence of foam indicates the presence of saponins.

#### 1.4.6 Test for steroids

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. Two ml of chloroform was added followed by 1 ml of H<sub>2</sub>SO<sub>4</sub>. The formation of the reddish-brown ring at interface indicates the presence of steroids.

#### 1.4.7 Test for tannins

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. Two ml of 10% ferric chloride (FeCl<sub>3</sub>) was added into the test tube. The formation of dark blue or greenish-grey colouring indicates the presence of tannins.

#### 1.4.8 Test for anthocyanin and betacyanin

Plant extract (0.5 g) was dissolved into 2 ml MeOH into a test tube. One ml of 2 N NaOH was added and heated for 5 min at 100°C. The formation of bluish-green colour indicates the presence of anthocyanin, and the formation of yellow colour indicates the presence of betacyanin.

#### 1.4.9 Test for coumarins

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. The test tube was covered with filter paper which was moistened with 1 ml NaOH. The test was placed in boiling water for a few minutes. Then the filter paper was removed, and examined under the UV light for yellow fluorescence to indicate the presence of coumarins.

#### 1.4.10 Test for carbohydrates (Fehling's Test)

Plant extract (0.5 g) was dissolved into 2 ml of methanol in a test tube. One ml of Fehling's A and 1 ml of Fehling's B was added into the test tube. Test tubes were placed in a boiling water bath for a few minutes, and test tube content was mixed as they come to boiling, and colour change and precipitate formation was observed. Formation of a yellow or brownish-red precipitate indicates the presence of reducing sugars.

#### 1.4.11 Test for quinones

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. One ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added into a test tube. The formation of red colour indicates the presence of quinones.

#### 1.4.12 Test for terpenoids

Plant extract (0.25 g) was dissolved into 1 ml MeOH into a test tube. Two ml of chloroform was added into the test tube, and followed by 1.5 ml concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added. The formation of reddish-brown colour at the interface indicates the presence of terpenoids.

### 1.5 Determination of total phenolic content

The total phenolic content was evaluated by Folin Ciocalteu's method. One ml of plant extract (50, 150, 300, 450 µg/ml), and standard gallic acid (50, 100, 150, 300, 400, 450 µg/ml) was placed in the test tube, and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 min, 1.5 ml of 20% of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added and made up to 10 ml with distilled water. This was incubated for 2 h at room temperature, and intense blue colour developed. After incubation, absorbance was measured at 750 nm with a spectrophotometer (Anthos Zenyth 200 UV/Vis spectrophotometer). The experiment was performed in triplicates. The blank was done using reagent blank with solvent. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of plant extracts were expressed as mg of gallic acid equivalent weight (GAE)/100 g of dry weight (Samidha *et al.*, 2014, Aryal *et al.*, 2019).

### 1.6 Determination of total flavonoid content

The total flavonoid content was evaluated with the aluminium chloride colourimetric assay. One ml of plant extract (50, 150, 300, 450 µg/ml) and 1 ml of standard quercetin solution (50, 100, 150, 300, 400, 450 µg/ml) was added into test tubes and followed by 4 ml of distilled water, and 0.3 ml of 5% sodium nitrite solution. After 5 min, 0.3 ml of 10% aluminium chloride was added. At the 6<sup>th</sup> min, 2 ml of 1 M sodium hydroxide was added. The final volume was adjusted to 10 ml with distilled water and mixed well. The formation of orange yellowish colour was observed. The absorbance was measured at 510 nm with Athos Zenyth 200 UV/Vis spectrophotometer. The plant experiment was performed in triplicates. The blank was done using distilled water. Quercetin was used as a standard. The calibration curve was plotted using standard quercetin. The data of total flavonoids of plant extracts were expressed as mg of quercetin equivalents to 100 g of plant dry mass (Aryal *et al.*, 2019, Chandra *et al.*, 2014).

### 1.7 *In vitro* antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical-scavenging activity was carried out using the methods previously described by (Alara *et al.*, 2017). Essentially 0.3 mM of DPPH was prepared fresh in ethanoic solution, and 10 mg of each plant extract in various concentrations (10, 30, 40 and 50 mg/ml) was dissolved in 2 ml of methanol. Thereafter, 0.1 ml of prepared plant extract was added into a test tube containing 1.9 ml of DPPH radical solution. The mixtures were vortexed thoroughly and incubated in room temperature in the dark for 30 min. The absorbance was measured at 515 nm using Anthos Zenyth 200 UV/Vis spectrophotometer. Methanol was used as a blank. Ascorbic acid (Sigma-Aldrich, Johannesburg, South Africa) was used as a standard drug. The percentage of DPPH inhibition was evaluated using an equation that was previously described by (Omede *et al.*, 2018).

$$(\%) \text{ inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

The extracts with a scavenging activity higher than 80% were considered active. For extracts that had scavenging activity ranged between 50% and below 80% inhibition were considered moderate, and inactive were extract with <50% inhibition, and those inactive were rejected.

### 1.8 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay

The ability of selected plant extracts to scavenge free radical activities was determined by ABTS radical decolourization assay. The assay was previously described by (Re *et al.*, 1999), and it was further modified for this

study. Briefly, ABTS+ cation radical was produced by the reaction between 7 mM ABTS in water, and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12 – 16 h before use. ABTS+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl plant extract to obtain 3.995 ml of diluted ABTS+ solution, the absorbance was measured at 30 min after the initial mixing. The methanol solvent blank was run in each assay. The ABTS scavenging capacity of the extract was compared to butylated hydroxytoluene (BHT). The percentage inhibition of absorbance at 734 nm was calculated using the formula here below:

$$\text{Scavenging capacity (\%)} = \frac{100 - [\text{OD}_{\text{sample}} - \text{OD}_{\text{sample blank}}] \times 100\%}{\text{OD}_{\text{control}} - \text{OD}_{\text{control blank}}}$$

Where OD represents optical density or absorbance.

### 1.9 Alpha-amylase ( $\alpha$ -amylase) inhibition assay

Different concentrations (50, 150, 250, 350, 450 µg/ml) of each plant extract (500 µl) were incubated with 500 µl of  $\alpha$ -amylase enzyme solution (2 units/ml) was obtained by dissolving 0.001 g of  $\alpha$ -amylase in 100 ml of 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride) at room temperature (32°C) for approximately 10 min. After incubation period this was followed by the addition of 500 µl of 1% starch solution (dissolving 1 g of potato starch in 100 ml of distilled water with boiling and stirring for 15 min) and incubated at room temperature (32°C) for further 10 min. After the incubation period, 1 ml of 3,5-dinitro salicylic acid (DNSA) reagent was obtained by dissolving 1 g of DNSA in 50 ml of distilled water and added 30 g of sodium potassium tartrate in small lots, and the solution turns milky yellow. In the mixture above, 20 ml of 2 N sodium hydroxide was added and it turns transparent orange-yellow colour and this was made up to a mark of 100 ml in a volumetric flask. The reagent was wrapped with foil and kept in the dark was added to stop the reaction and was incubated in a hot water bath (85°C) for 5 min. The reaction was observed after 5 min' incubation time where the colour changed to orange-red and was removed from the water bath and cooled to room temperature.

The mixture was further diluted with 5 ml distilled water. The plant extracts in different concentrations were performed in triplicates. Individual blank was performed by replacing an enzyme with a buffer. Controls were performed by replacing plant extracts with solvent. Acarbose™ (500 µg/ml) was used as positive controls in the following concentrations (100, 200, 300, 400 and 500 µg/ml). Absorbance was measured at 540 nm with Anthos Zenyth 200 UV/Vis spectrophotometer. The percentage inhibition of  $\alpha$ -amylase was analyzed using the equation here below:

$$\% \text{inhibition} = \frac{(A_{540 \text{ Control}} - B_{540 \text{ Extract}})}{A_{540 \text{ Control}}} \times 100$$

## II. STATISTICAL ANALYSIS

The experimental results were expressed as mean  $\pm$  standard deviation of three parallel measurements. Linear regression analysis was used to calculate the IC<sub>50</sub> values. Significance difference (\*\*p < 0.05) and (\*\*\*)p < 0.0001) was determined using student "t" test by one-way ANOVA, Tukey's multiple comparison test, and Dunnett test in Prism version 5.0.

## III. RESULTS

The purpose of this study was to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha ( $\alpha$ )-amylase potential on the selected South Africa plant extracts. Thirteen phytochemicals were screened using standardized methods (Table 2). Furthermore, the determination of total phenol content and total flavonoid content were conducted in this study (Fig. 1, Fig. 2, and Table 3). In addition to this, the ability of plant extracts to scavenging free radicals was evaluated using DPPH radical scavenging assay (Fig. 3 and Fig. 4), and ABTS radical scavenging assay (Fig. 5). Finally, the ability of plant extracts to inhibit  $\alpha$ -amylase (Fig. 6) was also evaluated.

### 4.1 Qualitative phytochemicals

The qualitative phytochemicals investigations are showed the presence of phytochemicals in various concentrations, and the results are summarised in Table 2 here below.

**Table 2:** Qualitative analysis of phytochemicals results of nine plant extracts.

Plant extracts	Part	<sup>a</sup> ALK	<sup>b</sup> CAR	<sup>c</sup> FLA	<sup>d</sup> PHE	<sup>e</sup> SAP	<sup>f</sup> STE	<sup>g</sup> TA	<sup>h</sup> ANT	<sup>i</sup> BEC	<sup>j</sup> COU	<sup>k</sup> CRB	<sup>m</sup> QUI	<sup>n</sup> TEP
<i>Euclea crispa</i>	L	-	-	+	+	+	-	+	+	-	+	-	+	+
<i>Euclea natalensis</i>	L	-	-	+	+	+	-	+	+	-	+	+	+	+
<i>Schkuhria pinnata</i>	L	+	-	+	+	-	-	-	-	+	+	-	+	+
<i>Ziziphus mucronata</i>	L	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>Ziziphus mucronata</i>	F	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>Lippia javanica</i>	L	+	+	+	+	+	+	+	-	+	+	-	-	+
<i>Vernonia oligocephala</i>	L	+	-	+	+	+	+	+	+	-	+	-	-	+
<i>Clerodendrum myricoides</i>	L	+	+	+	+	+	+	+	+	-	+	-	-	+
<i>Erythrina lysistemon</i>	L	+	+	+	+	-	+	+	+	-	+	-	+	+

L: Leaf; F: fruit; <sup>a</sup>ALK: Alkaloids; <sup>b</sup>CAR: Cardiac glycosides; <sup>c</sup>FLA: flavonoids; <sup>d</sup>PHE: phenols; <sup>e</sup>SAP: saponins; <sup>f</sup>STE: steroids; <sup>g</sup>TA: tannins; <sup>h</sup>ANT: anthocyanin; <sup>i</sup>BEC: Bectacynin; <sup>j</sup>COU: coumarins; <sup>k</sup>CRB: carbohydrates; <sup>m</sup>QUI: quinones; <sup>n</sup>TEP: terpenoids; +: present and -: absent.

### 5.2 The determination of total phenolic content

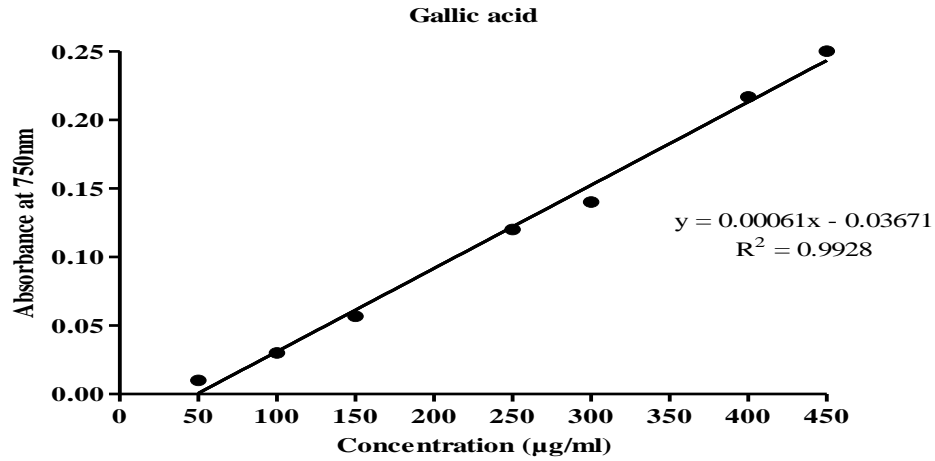


Fig. 1: Total phenolic content for standard gallic acid.  $R^2$  value represented mean data of  $n=3$

### 4.2 The determination of total flavonoids

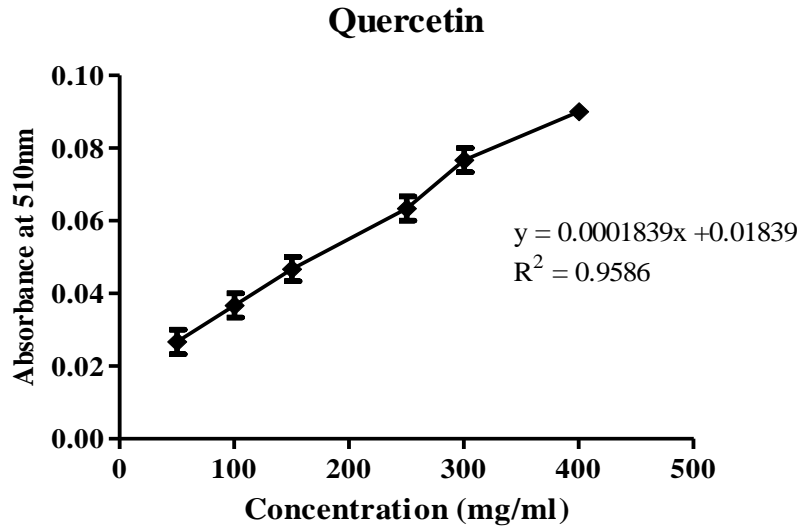


Fig. 2: Total flavonoids content for standard quercetin.  $R^2$  value represents the mean data set of ( $n=3$ )



**Table 3: Total phenolic content, and total flavonoids content of the selected South African plant extracts.**

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
<i>Euclea crispa</i>	Leaf	50	36,60± 0.94	4,60 ± 0.94
		100	46,19± 1.41	10,90 ± 0.94
		150	57,79± 0.94	32,95 ± 0.94
		250	70,39± 1.41	36,43 ± 1.41
		300	71,99± 2.36	38,80 ± 1.41
		400	82,58± 0.94	49,26 ± 0.94
		450	93,18± 1.41	61,05 ± 2.36
<i>Euclea natalensis</i>	Leaf	50	13,51± 0.94	6,18 ± 0.94
		100	36,67± 0.94	12,81 ± 1.41
		150	61,75± 1.41	16,75 ± 1.41
		250	72,26± 0.94	32,50 ± 1.41
		300	82,77± 0.94	48,24 ± 1.41
		400	93,28± 1.41	64,33 ± 1.89
		450	93,80± 1.89	79,74 ± 1.41
<i>Schkuhria pinnata</i>	Leaf	50	1,93± 0.47	3,70 ± 1.89
		100	7,72± 0.47	18,39 ± 0.94
		150	21,23± 1.41	34,09 ± 1.41
		250	38,60± 0.47	49,11 ± 0.94
		300	44,39± 0.94	64,81 ± 1.41
		400	50,18± 1.89	80,17 ± 1.41
		450	55,96± 1.41	82,34 ± 1.41

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
<i>Ziziphus mucronata</i>	Leaf	50	7,72± 0.47	2,24 ± 0.94
		100	19,30± 1.41	3,70 ± 1.89
		150	30,88± 1.89	4,60 ± 0.94
		250	36,67± 0.94	5.39 ± 0.94
		300	47,25± 0.94	26,45 ± 1.41
		400	57,83± 1.41	47,17 ± 1.41
		450	68,40± 0.94	67,56 ± 0.94
		<i>Ziziphus mucronata</i>	Fruits	50
100	25,09± 1.41			3,36 ± 1.41
150	46,32± 0.94			4,60 ± 0.94
250	55,97± 1.41			7,75 ± 0.94
300	66,54± 0.94			34,02 ± 1.41
400	77,12± 1.41			60,29 ± 1.89
450	87,70± 1.41			65,56 ± 0.94
<i>Lippia javanica</i>	Leaf			50
		100	5,79± 0.94	4,94 ± 1.41
		150	7,72± 0.47	20,87 ± 1.41
		250	21,23± 1.41	36,81 ± 1.41
		300	34,74± 0.94	53,08 ± 1.89
		400	48,25± 0.94	68,69 ± 1.41
		450	61,76± 1.41	74,62 ± 1.41

Plant species	Plant parts	Concentrations (µg/ml)	TPC (mg GAE/g) *	TFC (mg of quercetin equivalent/g dry material)
<i>Vernonia oligocephala</i>	Leaf	50	1,93± 0.47	2,91 ± 1.89
		100	3,86± 0.94	23,48 ± 1.89
		150	5,79± 0.47	44,06 ± 1.89
		250	5,72± 0.94	64,30 ± 1.41
		300	23,44± 1.41	74,88 ± 1.41
		400	39,16± 1.41	79,88 ± 1.41
		450	54,88± 0.94	85,45 ± 1.41
		<i>Clerodendrum myricoides</i>	Leaf	50
100	7,72± 0.94			3,70 ± 1.89
150	11,58± 1.41			19,06 ± 1.89
250	19,30± 1.41			34,09 ± 1.41
300	35,02± 1.41			49,78 ± 1.89
400	50,74± 1.89			64,14 ± 0.47
450	66,46± 0.94			80,51 ± 1.89
<i>Erythrina lysistemon</i>	Leaf			50
		100	42,46± 1.89	7,30 ± 1.41
		150	52,91± 1.89	23,48 ± 2.36
		250	63,37± 1.41	38,66 ± 1.89
		300	73,82± 0.47	54,17 ± 1.89
		400	84,28± 2.83	70,02 ± 2.36
		450	94,74± 0.94	75,87 ± 1.41

\*all values are expressed as mean ± standard error means (SEM) (n=3), TPC = Total phenolic content, and TFC = Total flavonoids content.

### 4.3 *In vitro* antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical

The determination of antioxidant activity from nine plant extracts were evaluated using DPPH radical scavenging activity. The standard curve of ascorbic acid was measured and shown in Fig. 3. The results are expressed in percentage of inhibition; of which active extract was above 50% and anything below 50% was considered inactive and rejected. Four active plant extracts are summarised (Fig. 4) here below. One-way ANOVA was used to establish statistical significance between plant extracts and concentrations used. A degree of significance was observed with one or two concentrations used. The inhibition effects that were observed for *Schkuhria pinnata* showed 95% inhibition (50 mg/ml) and *Lippia javanica* showed 95% inhibition (50 mg/ml) *Clerodendrum myricoides* showed 93% inhibition (50 mg/ml), and *Erythrina lysistemon* showed 92% inhibition (50 mg/ml), respectively. The significant difference between groups was observed  $** (P < 0.005)$  between *S. pinnata* vs *C. myricoides*, *S. pinnata* vs *E. lysistemon*, and *L. javanica* vs *E. lysistemon*. It can be further seen that the inhibition has a dose- respond.

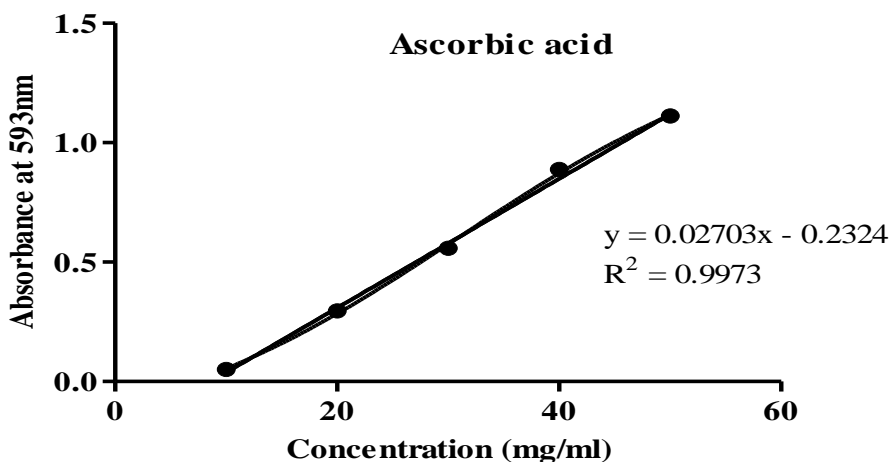


Fig. 3: *In vitro* antioxidant (DPPH) assay for standard Ascorbic acid.  $R^2$  values represented mean data set of  $n=3$

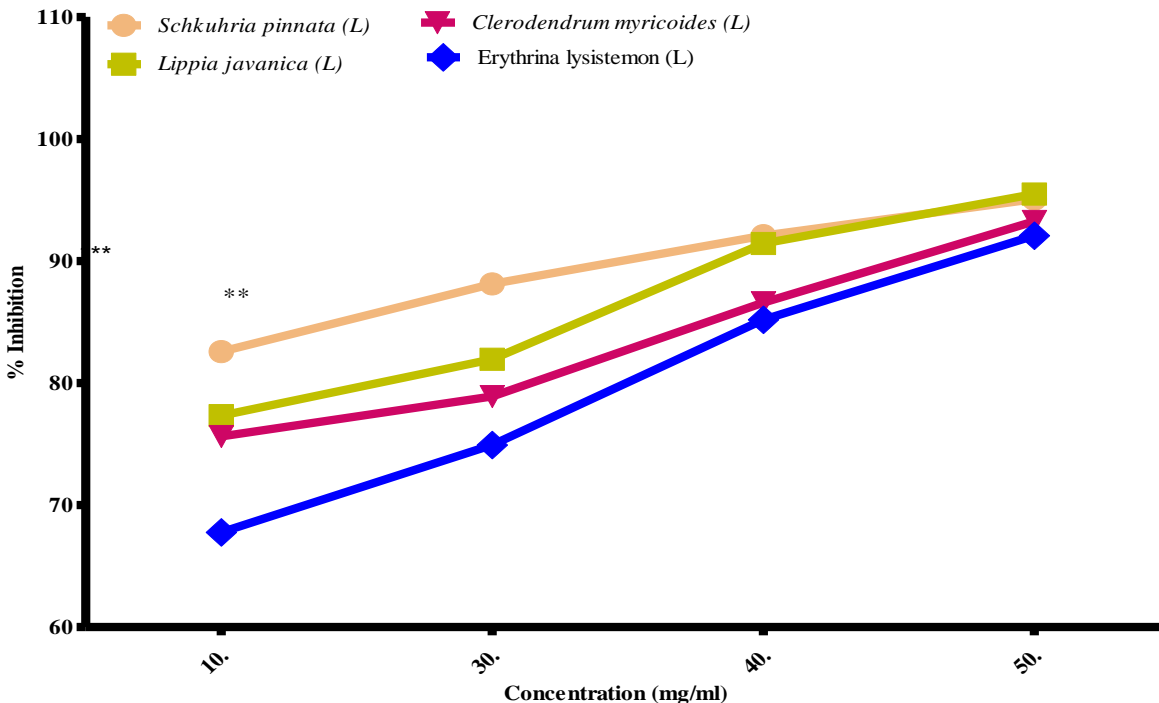
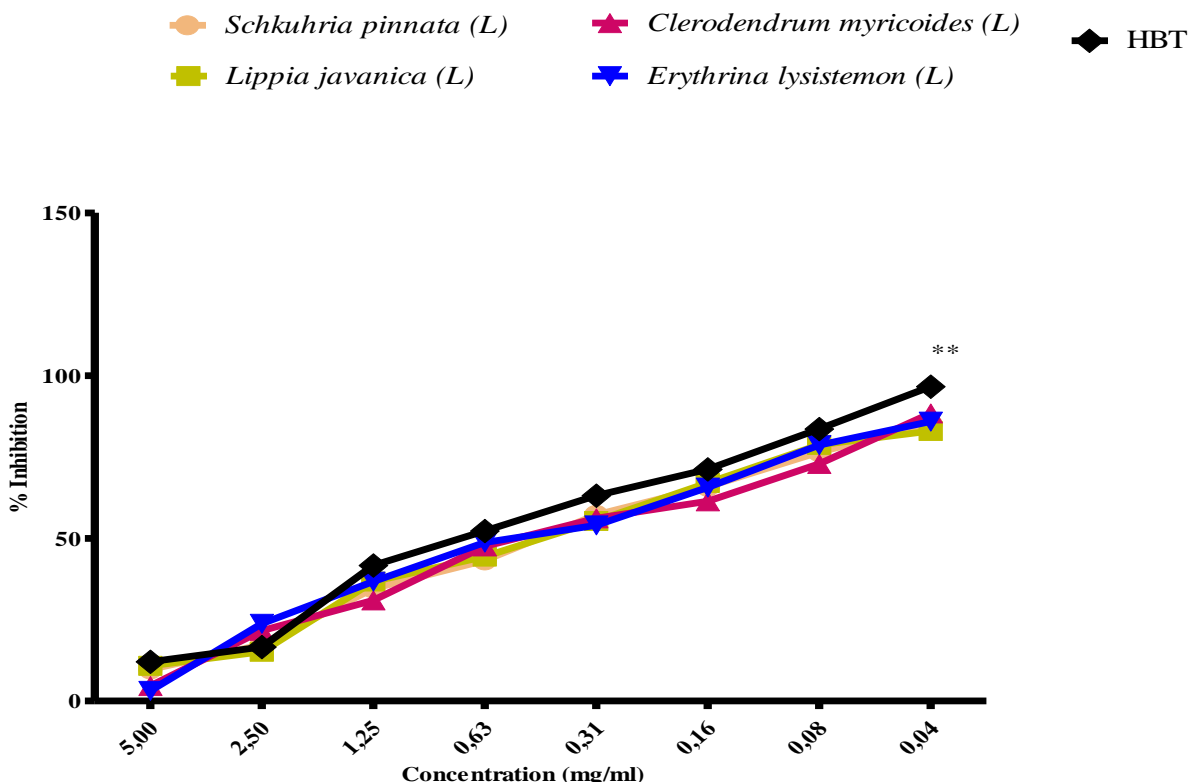


Fig. 4: DPPH scavenging activities for *Schkuhria pinnata* ( $IC_{50} = 38.244$  mg/ml), *Lippia javanica* ( $IC_{50} = 34.966$  mg/ml), *Erythrina lysistemon* ( $IC_{50} = 37.828$  mg/ml), and *Clerodendrum myricoides* ( $IC_{50} = 38.939$  mg/ml). L: Leaf, and F: fruit. (Significant,  $**p < 0.005$ ).

#### 4.4 ABTS radical scavenging activity assay

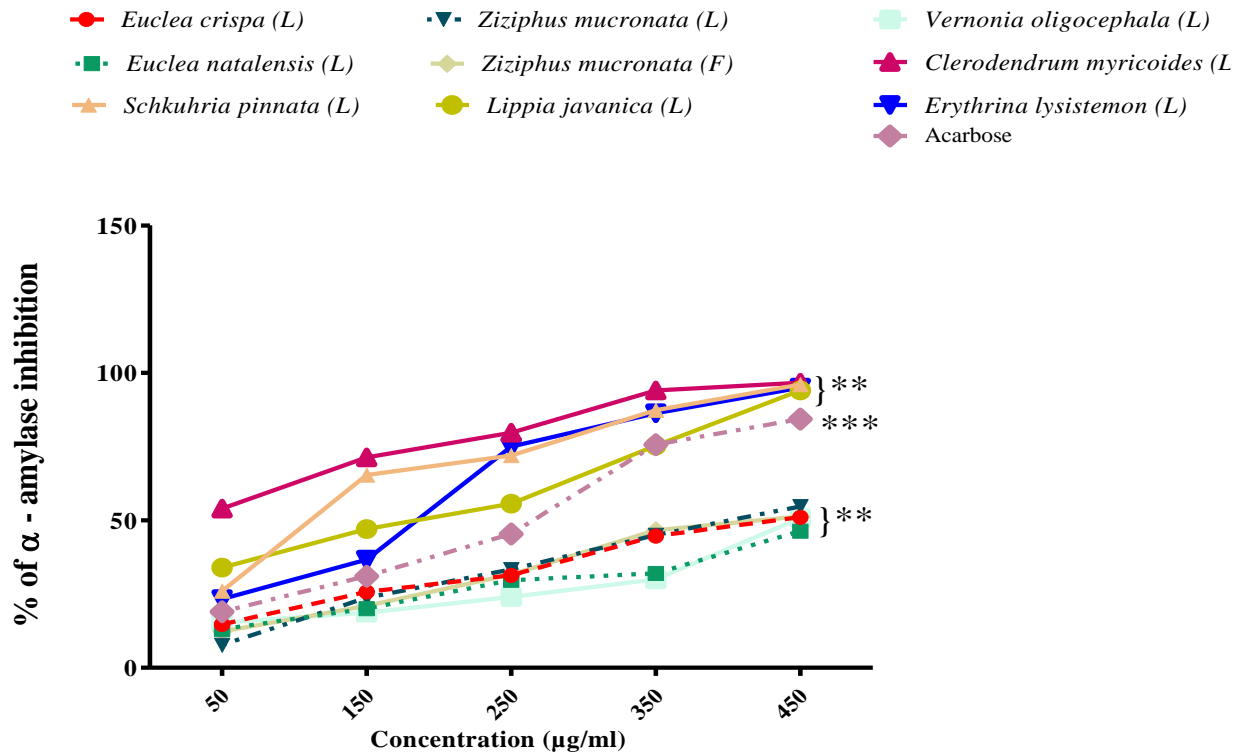
The four plant extracts that demonstrated good scavenging activity against DPPH radical assay were further validated with ABTS and similar results were observed (Fig. 5). The inhibition effects and IC<sub>50</sub> value were observed for *Clerodendrum myricoides* showed 88% inhibition (0.04 mg/ml), and IC<sub>50</sub> value of 7.148 mg/ml, for *Erythrina lysistemon* showed 86% inhibition (0.04 mg/ml), and IC<sub>50</sub> value of 5.353 mg/ml *Schkuhria pinnata* showed 85% inhibition (0.04 mg/ml), and IC<sub>50</sub> value of 0.944 mg/ml and *Lippia javanica* showed 83% inhibition (0.04 mg/ml), and IC<sub>50</sub> value of 0.759 mg/ml. The standard inhibitor BHT showed 97% (0.04 mg/ml). The significant difference between plant extracts and BHT was observed to be **\*\***(p<0.05).



**Fig. 5:** ABTS scavenging activities for *Schkuhria pinnata* (IC<sub>50</sub> = 0.944 mg/ml), *Lippia javanica* (IC<sub>50</sub> = 0.759 mg/ml), *Erythrina lysistemon* (IC<sub>50</sub> = 5.353 mg/ml), and *Clerodendrum myricoides* (IC<sub>50</sub> = 7.148 mg/ml). L: Leaf, and F: fruit. (Significant between groups and control (HBT (IC<sub>50</sub> = 2.763 mg/ml)), **\*\***(p<0.005).

#### 4.5 In vitro inhibitory α-amylase assay

Nine selected plant extracts were used to assess the ability of α-amylase inhibitory effects and were compared with a known inhibitor (Acarbose™). A degree of α-amylase was observed in all plant extracts which ranged from 9% to 98%. However, only four plant extracts demonstrated a good inhibition effect, and similar results were observed for Acarbose™. Essentially, plant extracts were further quantified by chromogenic DNSA method and good inhibition effects was observed and lower IC<sub>50</sub> values for *Schkuhria pinnata* (IC<sub>50</sub> = 52.673 μg/ml), and *Clerodendrum myricoides* (IC<sub>50</sub> = 322.287 μg/ml) showed 98% inhibition (450 μg/ml), *Erythrina lysistemon* (IC<sub>50</sub> = 205.089 μg/ml) showed 97% inhibition (450 μg/ml), and *Lippia javanica* (IC<sub>50</sub> = 247.708 μg/ml) showed 96% inhibition (450 μg/ml). The standard inhibitor Acarbose™ (IC<sub>50</sub> = 291.395 μg/ml) showed 87% (450 μg/ml). The significant difference was observed between groups **\*\***(p<0.05) and between groups and control **\*\*\*** (p<0.0001).



**Fig. 6:** α-amylase inhibition activity of Acarbose™ and nine selected plant extracts. L: Leaf, and F: fruit. The four plant extract exhibited good α-amylase inhibition and lower IC<sub>50</sub> values were measured for *Schkuhria pinnata* (IC<sub>50</sub> = 52.673 µg/ml), and *Clerodendrum myricoides* (IC<sub>50</sub> = 322.287 µg/ml) showed 98% inhibition (450 µg/ml), *Erythrina lysistemon* (IC<sub>50</sub> = 205.089 µg/ml) showed 97% inhibition (450 µg/ml), and *Lippia javanica* (IC<sub>50</sub> = 247.708 µg/ml) showed 96% inhibition (450 µg/ml). The standard inhibitor Acarbose™ (IC<sub>50</sub> = 291.395 µg/ml) showed 87% (450 µg/ml). The (Significant between groups was observed to be \*\*p<0.05, and for control vs groups to be \*\*\*p<0.0001).

## 2. discussion

This study aimed to evaluate preliminary phytochemicals, total phenolic content, total flavonoid content, antioxidants, and alpha (α)-amylase potential on the selected South Africa plant extracts. In qualitative analysis, the phytochemicals that were analysed ranged from 7 to 10 out of 13 tested phytochemicals identified. The total phenolic content was determined and results are shown (Fig. 1, and Table 3). For total flavonoid content results are shown (Fig. 2, and Table 3). Four plant extracts demonstrated scavenging activities above 80%, which are *Schkuhria pinnata*, *Lippia javanica*, *Clerodendrum myricoides*, and *Erythrina lysistemon* (Fig. 4, and Fig. 5). Similarly, these plant extracts also exhibited α-amylase inhibition effects of up to 98% (Fig. 6).

### 5.1 Qualitative phytochemicals

The qualitative phytochemicals investigations conducted on selected plant extracts showed the presence of various phytochemicals constitutes. All plant extracts indicated the presence of flavonoids, phenolics, coumarins, and terpenoids. Alkaloids were not present in *Euclea crispa*, and *Euclea natalensis*. Cardio glycosides were present in *Lippia javanica*, *Clerodendrum myricoides*, and *Erythrina lysistemon*. Saponins were present in *Euclea crispa*, *Euclea natalensis*, *Vernonia oligocephala*, *Lippia javanica*, and *Clerodendrum myricoides*. Steroids were present in *Lippia javanica*, *Veronia oligocephala*, *Clerodenrum myricoides*, and *Erythrina lysistemon*. Tannins were present in all plant extracts, except *Schkuhria pinnata*. Bectaynin was present in *Schuhria pinnnata*, *Ziziphus mucronata* (leaf and fruits), and *Lippia javanica*. Anthocynin were absent in *Schkuhria pinnnata*, and *Lippia javanica*. Carbohydrates were present in *Euclea natalensis* and *Ziziphus mucronata* (leaf and fruits). Quinones was not present in *Lippa javanica*, *Vernonia oligocephala* and *Clerodendrum myricoides*. The phytochemicals that have been identified in this study have been extensively studied and their uses in literature.

Flavonoids are naturally found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Panche *et al.*, 2016, Ali, 2011). Interestingly, flavonoids play an important role in the following industries such as nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al.*, 2016). In recent research has shown that flavonoids contribute towards anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Kumar and Pandey, 2013, Ayaz *et al.*, 2019).

The phenolics are organic compounds and are found in various plant species, and have protective effects against infection and herbivory by an insect (Ali, 2011). The protective effects attribute to anti-inflammatory and antiseptic and can have anti-viral properties (Cianciosi *et al.*, 2018, Shahidi and Yeo, 2018). Coumarins an aromatic organic [chemical compound](#) that is colourless crystalline solid with a sweet odour resembling the scent of [vanilla](#) and a bitter taste (Jain and Joshi, 2012). They are naturally found in various plant species and can serve as a defence against predators (Yamane *et al.*, 2010). Coumarins have been reported to possess the biological activity and have limited approval for a few medical uses as pharmaceuticals, such as in the treatment of [lymphedema](#) (Musa *et al.*, 2008). The terpenoids are natural chemical compounds that are present in all living organisms (Pichersky and Raguso, 2018). Terpenoids are found in abundance mostly in green plants and, particularly, flowering plants, when compared with living organisms (Bergman *et al.*, 2019). Essentially, terpenoids have a variety of functions such as hormones, components of electron transfer systems, protein modification agents, membrane fluidity determinants, and antioxidants (Tan *et al.*, 2018, Bergman *et al.*, 2019).

Alkaloids are naturally occurring in [plants](#), and more common in certain families of flowering plants (Hussain *et al.*, 2018). The most common alkaloids are morphine, codeine, ergonive, and ephedrine (Sayhan *et al.*, 2017, Harborne, 1993). They provide protective effects on plants against predators such as insects (War *et al.*, 2012, Furstenberg-Hagg *et al.*, 2013). Furthermore, alkaloids have diverse medicinal properties such as relief of pain (morphine) (Shoaib *et al.*, 2016, Hussain *et al.*, 2018), treat analgesic (codeine) (Bhandari *et al.*, 2011), treatment of arrhythmias (quinidine) (Eyal, 2018), and useful for blood-vessel constrictors (ergonovine and ephedrine) (Hartmann, 2004). In addition to this, ergonovine is used to reduce uterine haemorrhage after birth (Weeks, 2015, Chelmwow, 2008), and finally, ephedrine has been used often for the relief of discomfort associated with common colds, sinusitis, hay fever, and bronchial asthma (Thacher, 1946). Cardiac glycosides are a class of organic compounds that are found in various medical plants (Musa *et al.*, 2008). The compounds that have been isolated from cardio glycosides such as digitoxin, digoxin, and convallotoxin have been reported to support heart strength and rates of contraction (Grosso *et al.*, 2017, Ambrosy *et al.*, 2014). Moreover, cardio glycosides have an additional function which is a diuretic effect that stimulates urine production and aids in the removal of fluid from tissues and the circulatory system (Mladenka *et al.*, 2018). Essentially, cardio glycosides are also used treatments for [congestive heart failure](#), and [cardiac arrhythmias](#) (Mashour *et al.*, 1998).

Saponins are a class of chemical compounds that are widely found in varies plant species (Faizal and Geelen, 2013). Researchers have investigated numerous properties of including beneficial and detrimental effects on human health, pesticidal, insecticidal, molluscicidal and fungicidal activity, bitterness and sweetness and other industrial applications such as foaming and surface-active agents (Wagner, 2000, Kharkwal *et al.*, 2012, Wisetkomolmat *et al.*, 2019, Bossou *et al.*, 2013). The steroids are biologically active [organic compounds](#), which are found in various plants, animals and fungi (Dembitsky *et al.*, 2017). The biological function of steroids is to alter [membrane fluidity](#), and also molecule signalling (Dufourc, 2008). Steroids are hormones that play an important role in the alterations in anatomy and physiology during important developmental (Zubeldia-Brenner *et al.*, 2016).

Tannins are polyphenolics which are produced by plants and are bitter (El Gharras, 2009). The bitterness in tannins serves as a protective mechanism against predators such as insects and grazing animals (War *et al.*, 2012). In addition to this, tannins are also useful in curing leather because they tend to contract and astringe tissues by binding with precipitating proteins (Ashok and Upadhyaya, 2012). Anthocyanins are [water-soluble](#) and they can either have a red, blue or black pigment which is found in plants and vegetables (Archetti *et al.*, 2009). Anthocyanin is usually found on the blueberry, raspberry, [black rice](#), and black soybean (Khoo *et al.*, 2017). The importance of plants with pigmentations is mainly for signalling, as in attracting pollinating and dispersal agents and repelling herbivores (Lee, 2005). Anthocyanins are used in the food industry as an additive and possess anti-oxidant properties (Khoo *et al.*, 2017). Similarly, bectacyanin are yellow indole-derived pigments found in plants (Khoo *et al.*, 2017). They are commonly noticeable in the petals of flowers but may colour the fruits, leaves, stems, and roots of plants that contain them (Khoo *et al.*, 2017, Miguel, 2018) Essentially they are used as additive as a [food dye](#) and they have been observed to possess [antioxidants](#) (Miguel, 2018).

Carbohydrates are commonly found in organisms and plants (Ainsworth and Bush, 2011). They are among the first organic compounds formed during photosynthesis (Ainsworth and Bush, 2011). The key function of carbohydrates is a source of energy by all cells (Jéquier, 1994). Also, carbohydrates can be used for the production of cellulose in cell walls, for the synthesis of storage products such as starch (Stein and Granot, 2019). The quinones are secondary metabolites that are found in various plant species (Lu *et al.*, 2013). Quinones possess biological activities, such as the purgative effect of as well as the antibacterial and anti-cancer activities (Koyama *et al.*, 2010, Bolton *et al.*, 2000). Moreover, quinine has been instrumental in the derivatives of various natural and artificial colouring substances ([dyes](#) and [pigments](#)) (Yusuf *et al.*, 2017).

## 5.2 The determination of total phenolic content

The total phenolic content was estimated by Folin Ciocalteu's method using gallic acid as standard. The reagent was formed after mixing phosphotungstic acid, and phosphomolybdic acid which after oxidation of the phenolic is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue colouration produced has maximum absorption in the region of 750 nm and proportional to the total quantity of phenolic compounds originally present. The gallic acid solution of concentration (50 – 450 µg/ml) conformed to Beer's Law at 750 nm with a regression coefficient ( $R^2$ ) = 0.9928. the plot has a slope (m) = 0.00061 and intercept = 0.03671. The equation of standard curve is  $y = 0.00061x + 0.03671$  (**Fig. 1**). In this study it was observed that plant extracts exhibited total phenolic



content, however, *Erythrina lysistemon* ( $94.71 \pm 0.94$  GAE/g), *Euclea crispa* ( $93.18 \pm 1.41$  GAE/g), *Euclea natalensis* ( $93.18 \pm 1.89$  GAE/g), *Ziziphus mucronata* ( $87.7 \pm 87.7$  GAE/g) exhibited high total phenolic in high concentration (450  $\mu$ g/ml).

### 5.3 The determination of total flavonoid content

The total flavonoid content was estimated using aluminium chloride colourimetric assay using quercetin as standard. Aluminium chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols. Besides, it also forms liable forms complexes with ortho dihydroxide groups in A/B rings of flavonoids. The quercetin solution of concentration (100 – 500 mg/ml) conformed to Beer's Law at 510 nm with a regression coefficient ( $R^2$ ) = 0.9586. The plot has a slope (m) = 0.0001839 and intercept = 0.01839. The equation of standard curve is  $y = 0.0001839x + 0.01839$  (Fig 2). The total flavonoid content was also observed to have relatively high results. Interestingly, no similar plant extracts exhibited similar results as total phenolic content, however, the following plants exhibited high total flavonoid content in higher concentrations (450  $\mu$ g/ml). The plant extracts are *Vernonia oligocephala* ( $85.45 \pm 1.41$  mgG/g), *Schkuhria pinnata* ( $82.34 \pm 1.41$  mgG/g), and *Clerodendrum myricoides* ( $80.51 \pm 1.89$  mgG/g).

### 5.4 In vitro antioxidant assay - 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity was determined using DPPH radical scavenging assay and ascorbic acid was used as standard. The ascorbic acid solution of concentration (10 – 50 mg/ml) conformed to Beer's Law at 593 nm with the regression coefficient ( $R^2$ ) = 0.9973. The plot has a slope (m) = 0.02703 and intercept = 0.2324. The equation of standard curve is  $y = 0.02703x - 0.2324$  (Fig. 3). The results were expressed as a percentage of inhibition and  $IC_{50}$  (where 50% concentration of the extract scavenged free radical). The lower the  $IC_{50}$  ( $\mu$ g/ml) value the higher the percentage of free radicals. The plant extracts with a scavenging activity higher than 80% were considered active. For extracts that had scavenging activity ranged between 50% and below 80% inhibition were considered moderate and inactive were extract with <50% inhibition, and those inactive were rejected. The antioxidant using DPPH radical scavenging activity revealed that four plant extracts scavenged free radicals namely: - *Schkuhria pinnata* ( $IC_{50} = 38.244$  mg/ml), *Lippia javanica* ( $IC_{50} = 34.966$  mg/ml), and *Erythrina lysistemon* ( $IC_{50} = 37.828$  mg/ml) (Fig. 4).

### 5.5 ABTS radical scavenging activity assay

The plant extracts that were considered active from DPPH were further verified with ABTS antioxidant assay. The results were expressed as percentage inhibition and  $IC_{50}$  (where 50% concentration of the plant extract scavenged free radical). Essentially, the lower  $IC_{50}$  (mg/ml) value the higher the percentage inhibition of free radicals. The antioxidant determined using ABTS radical scavenging activity reveals that *Schkuhria pinnata* ( $IC_{50} = 0.944$  mg/ml), *Lippia javanica* ( $IC_{50} = 0.759$  mg/ml), *Erythrina lysistemon* ( $IC_{50} = 5.353$  mg/ml), *Clerodendrum myricoides* ( $IC_{50} = 7.148$  mg/ml), (HBT ( $IC_{50} = 2.763$  mg/ml) (Fig. 5). The natural bioactive compounds such as antioxidants normal occur in medicinal plants and they play a pivotal role in scavenging free radicals (Babajide *et al.*, 2010, Kalaivani and Mathew, 2010). In this present study, four plant species exhibited high antioxidant activities in both DPPH and ABTS radical assays. These plant species can play a pivotal role in the prevention of various degeneration diseases, and these results confirm their use in traditional medicine practice purposes (Rice-evans *et al.*, 1995, Xu *et al.*, 2017).

### 5.6 In vitro inhibitory $\alpha$ -amylase assay

The plant extracts understudy demonstrated some degree of  $\alpha$ -amylase inhibition, however, a good inhibition was noticeable *Schkuhria pinnata* ( $IC_{50} = 52.673$   $\mu$ g/ml), and *Clerodendrum myricoides* ( $IC_{50} = 322.287$   $\mu$ g/ml) showed 98% inhibition (450  $\mu$ g/ml), *Erythrina lysistemon* ( $IC_{50} = 205.089$   $\mu$ g/ml) showed 97% inhibition (450  $\mu$ g/ml), and *Lippia javanica* ( $IC_{50} = 247.708$   $\mu$ g/ml) showed 96% inhibition (450  $\mu$ g/ml). The standard inhibitor Acarbose<sup>TM</sup> ( $IC_{50} = 291.395$   $\mu$ g/ml) showed 87% inhibition (450  $\mu$ g/ml) (Fig. 6).

The oxidative damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes, and other chronic health conditions (Halliwell, 1994, Diplock *et al.*, 1998, Valko *et al.*, 2007, Oyedemi *et al.*, 2017) plays a significant role in the pathogenesis of metabolic syndrome including diabetes mellitus (DM). The ability of medicinal plants to inhibit  $\alpha$ -amylase is an important therapeutic target in the regulation of postprandial increase of blood glucose in diabetic patients (Oyedemi *et al.*, 2017). Importantly, the plant species that exhibited good  $\alpha$ -amylase inhibition activity can be a candidate for use in the blood glucose regulation in diabetic patients.

## IV. CONCLUSION

This study has shown the presence of medicinally important properties in plant species studied. The presence of phytochemicals in plant species shown the usefulness of these plant species in traditional medicine practices. Also, certain plant species in this study demonstrated antioxidant potential and  $\alpha$ -amylase inhibition potential was observed. The study suggests that the presence of phenolic, flavonoids, antioxidants, and  $\alpha$ -amylase properties are potential solutions towards the management of diabetes and other chronic inflammatory diseases. The results obtained in this study support the use of these plants in the management of various ailments in traditional medicine practices.



## V. CONFLICT OF INTEREST

The authors declare that they do not have any conflict concerning the publication of this paper.

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