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IN-VITRO ALPHA- AMYLASE INHIBITORY ACTIVITY, ANTIOXIDANT POTENTIAL, AND GC-MS ANALYSIS OF CREPE GINGER (*COSTUS SPECIOSUS* (J. KOENIG.) SM) LEAVES

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ABSTRACT: The leaves of crepe ginger (*Costus speciosus* (J. Koeng.) Sm. are utilized by the Subanen tribe of Lapuyan, Zamboanga del Norte, Philippines to treat diabetes. However, the hypoglycemic potential of this plant is not yet well-established. Thus, the present study investigated the *in-vitro* alpha-amylase inhibitory and antioxidant activities of the leaves of *C. speciosus*. The evaluation of the alpha-amylase inhibitory activity was conducted *in-vitro* using soluble starch. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was employed in the evaluation of antioxidant activity. Total phenolics content was investigated using Folin-Ciocalteu reagent. The presence of phytoactive compounds that might contribute to its activities was determined through phytochemical screening and Gas chromatography - Mass spectrometric analysis. The results revealed that the leaf extracts exhibited alpha-amylase inhibitory potential and antioxidant activity which might be due to the presence of bioactive compounds such as eicosane, nonadecane, squalene, phenol, 2, 4-bis (1, 1-dimethylethyl), d-limonene, and hexadecanoic acid, ethyl ester. Negative and low correlation between DPPH scavenging activity and total phenolics content were obtained from both extracts. Nevertheless, the extracts may serve as source of natural antidiabetic and antioxidant agent.

INTRODUCTION: Alpha-amylase (alpha-1, 4-glucan-4-glucanohydralase) is one of the major secretory products of the pancreas (about 5-6%) and salivary glands which play a significant role in the digestion of starch to oligosaccharides ^{1, 2, 3}. It can be found in microorganisms, plants, and higher organisms ⁴. By blocking the hydrolysis of 1, 4-glycosidic linkages of starch and other oligosaccharides into simple sugars, alpha- amylase inhibitors “starch blockers” prevent or slow down the absorption of starch ⁵.

Therefore, alpha-amylase inhibitors play a significant contribution to the treatment of disorders in the uptake of carbohydrates ⁶. If not inhibited, the rapid degradation of dietary starch by alpha-amylase results to elevated postprandial hyperglycemia leading to diabetes ⁷.

Diabetes, a multifactorial disease, is characterized by chronic hyperglycemia with disturbance or abnormality of carbohydrate, fat, and protein metabolism resulting from absolute or relative lack of insulin secretion and insufficient cellular effect of insulin ^{8, 9, 10} which might be due to aging, heredity, obesity, unbalanced diet, sedentary lifestyle, drugs, stress, hypertension, and / or pancreatic dysfunction ¹¹. Diabetes is often associated with some long-term diseases such as cardiovascular diseases and abnormalities in peripheral vascular, ocular, neurologic, and renal

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abnormalities which leads to morbidity and death¹². Lowering of postprandial hyperglycemia is one of the various therapeutic approaches^{13, 14} by impeding absorption of glucose through inhibition of glucose and carbohydrate-hydrolyzing enzyme such as alpha-amylase¹⁵. Several inhibitors of alpha-amylase have been isolated and investigated from medicinal plants to serve as an alternative drug with increased potency and have fewer side effects than the existing synthetic drugs. Plants are potent free radical neutralizer thus amends degenerative diseases such as diabetes¹⁶.

There has been a variety of plants studied which show alpha-amylase inhibitory activity and may be relevant to the treatment of type 2 diabetes¹⁷. Many natural plants such as *Amaranthus viridis* Linn., *Andrographis paniculata*, *Saurus chinesis*, and flowers of *Punica granatum* have been evaluated for their antidiabetic potential¹⁸. *C. speciosus* is one of the medicinally important plants belonging to family Zingiberaceae. Vijayalakshmi and Sarada¹⁹ examined the polyphenol content and antioxidant potential of the different parts of *C. speciosus*. The chloroform leaf extract showed antioxidant property²⁰. Aqueous extract was found to be effective in reducing obesity in albino rats²¹. Moreover, Sanadhya et al.,²² found that the methanolic leaf extract has antioxidant, anti-inflammatory, and anti-hemolytic activities, and further inhibits hemagglutination which is due to the presence of different phytoconstituents.

The rhizome of *C. speciosus* is extensively studied for its antidiabetic potential and antioxidant ability. Eremanthin, costulonide, saponins, triterpenes, and beta sitosterol are among the bioactive compounds isolated from the rhizome which contribute to its hypoglycemic potential by stimulating insulin secretion and tissue glucose uptake^{23, 24}. Thambi and Safi²⁵ found that the essential oil of rhizomes contains different sesquiterpenoids and among which α -humulene and zerumbone are already reported to have anticancer properties. In addition, they found that the essential oil has antimicrobial properties. Nehete et al.,²⁶ reported the antioxidant activity and phenolic content of the rhizome. *In-vivo* antihyperglycemic and antioxidant potential study was conducted by Bavarva and Narasimhacharya²⁷ and concluded that root extract has antihyperglycemic and antioxidative effects.

Despite the presence of numerous synthetic drugs, the rural areas of developing countries still opt for the utilization of natural products from medicinal plants for the treatment of diabetes due to the effectiveness, fewer negative side effects, and economic friendly nature^{28, 29}. The aqueous leaf extract of *C. speciosus* is being used in the treatment of diabetes in folklore medicine and by the Subanen tribe of Lapuyan. There have been studies conducted on different parts of this plant but the leaves are the less studied part and there is no study carried out on its alpha-amylase inhibitory activity. Due to the lack of scientific explanations on traditional knowledge, there is a demand for plant-based drug discovery to fill the gap and identify new health care strategies to combat multifactorial diseases such as diabetes. Thus, the present study was conducted to evaluate the anti-diabetic and antioxidant potential of hydroethanolic and aqueous leaf extracts of *C. speciosus*.

MATERIALS AND METHODS:

Plant Collection: The plant was selected based on its ethnopharmacological use in traditional medicine. The leaves of *C. speciosus* were collected from the Municipality of Lapuyan, Zamboanga del Sur. The leaves of *C. speciosus* (accession number NSM-1643) were washed well under running tap water and then with distilled water, air dried, powdered, and stored in an airtight container.

Preparation of Extracts:

Soxhlet Extraction: The leaves of *C. speciosus* were cut into pieces and air dried at room temperature. The leaves were ground into a powder using dry mechanical grinder. Ten grams (10.0 g) of powdered leaves were continuously extracted with 150.0 mL 95% ethanol using Soxhlet apparatus up to 48 h at 50 °C. The extract was filtered and concentrated in rotary evaporator at 40-50 °C under reduced pressure to obtain a semisolid material and was stored in a freezer for future use³⁰.

Decoction: Four hundred milliliters (mL) of distilled water were added to 15.0 g of powdered leaves of *C. speciosus*. The mixture was boiled to concentrate it to 100.0 mL. The decoction was then filtered through a vacuum pump using a Whatmann no. 1 filter paper. The obtained filtrates were

subjected to evaporation in a rotary evaporator at 50.0 °C. The resultant extract was lyophilized to remove excess water and was placed in air tight containers and stored in the freezer for further use.

Alpha-Amylase Inhibitory Activity: Total of 500.0 µL of test samples and acarbose (positive control) were added to 500.0 µL of 0.20 mM phosphate buffer (pH 6.9) containing alpha-amylase (0.5 mg/mL) solution and was incubated at 25 °C for 10 min. Five hundred microliters (µL) of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) were added to each tube. Reaction mixtures were incubated at 25 °C for 10 min and the reaction was stopped using 1.0 mL of 3, 5-dinitrosalicylic acid color reagent. Test tubes were then incubated in a boiling water bath for 5 min. and cooled to room temperature. The reaction mixture was diluted by adding 10.0 mL distilled water and absorbance was measured at 540 nm using UV-Vis spectrophotometer (SHIMADZU UV mini 1240). The control samples represent 100% enzyme activity and were prepared without any plant extract³¹.

DPPH Radical Scavenging Activity: Antioxidant activity of the *C. speciosus* leaf extracts was quantified by measuring the DPPH radical scavenging activity. The DPPH (Sigma) scavenging activity of extract was determined using spectrophotometry which was adopted from the method of Perera *et al.*,³². Reaction mixture was prepared using 2.5 mL of 6.5×10^{-5} M freshly prepared DPPH solution and 0.5 mL of extract dissolved in absolute methanol. Extracts were tested in four concentrations (0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL) with three replicates at room temperature under dark condition. Absorbance was measured at 540 nm using UV-Vis spectrophotometer (SHIMADZU UV mini 1240). Methanol was used as control. Ascorbic acid was used as the reference standard. The percentage of DPPH radical scavenging activity was determined using the equation:

$$\% \text{ scavenging activity} = (A_0 - A_s) / A_0 \times 100$$

Where, A_0 = Absorbance of the DPPH solution of the control.

A_s = Absorbance of the DPPH solution in the presence of plant extracts.

The total phenolics content of the extracts was carried out according to Formagio *et al.*,³³. Briefly, 100.0 µL of extract in methanol (1.0 mg/mL) was mixed with 1.0 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent (1:10 v/v). After mixing, 1.5 mL of 2% aqueous sodium bicarbonate was added, and the mixture was allowed to stand for 30 min with intermittent manual shaking. Total phenolics content is expressed as gallic acid equivalence (GAE) in mg gallic acid per gram of extract. The methanol solution served as blank. All assays were carried out in triplicate. The total phenolics content was determined from a standard calibration plot of absorbances of gallic acid measured at concentrations of 4.0 µg/mL, 8.0 µg/mL, 16.0 µg/mL, 32.0 µg/mL, 63.0 µg/mL.

Phytochemical Screening: To detect the presence of phytochemicals such as alkaloids, flavonoid, phenol, saponins, sterols, tannins, and terpenoids, the protocol of Ben *et al.*,³⁴ and Aguinaldo *et al.*,³⁵ was followed.

Test for the Presence of Alkaloids: To 1.0 mL of the extract, 2.0 mL of Wagner's reagent (iodine in potassium iodide) was added. A reddish brown colored precipitate indicates the presence of alkaloids.

Test for the Presence of Flavonoids: An equivalent of 10.0 g plant material was taken from the prepared extract, evaporated over a steam bath, and was cooled at room temperature. The extract was defatted with 9.0 mL hexane and water (2:1) by taking up a residue. The hexane fraction was discarded. The aqueous layer was diluted with 10.0 mL of 80% ethyl alcohol and filtered. The filtrate was placed in four test tubes. One portion was taken as control. The other three alcohol filtrates were separately treated with 0.5 mL of 12 M HCl and the color change was observed. The sample was warmed in a water bath for 15 min. Change in color was further observed within an hour and was compared with the control.

Test for the Presence of Phenols: A 200.0 µL of extract was mixed with 2.0 mL of distilled water and few drops of 5% ferric chloride were allowed to mix slowly along the sides of the tube. Appearance of dark blue color indicated the presence of phenols.

Test for the Presence of Saponins: A volume of extract equivalent to 2.0 g plant material was taken and was transferred into a test tube. Distilled water (10.0 mL) was added to tubes with a stopper. Tubes were shaken vigorously for 30 sec. The samples were allowed to stand for 10 min. and 'honeycomb' froth was observed. The result of the plant extract was compared with the standard. A 'honeycomb' froth greater than 2.0 cm height from the surface of the liquid after 10 min denoted a positive result.

Test for the Presence of Steroids: To the defatted aqueous extract, free from hexane, 3.0 mL of ferric chloride was added. The solution was stirred and 1.0 mL of concentrated H₂SO₄ was added cautiously from a pipette to a test tube in an inclined position. The mixture was allowed to stand upright and coloration at the interface of the acid and the aqueous layer was observed. A reddish-brown color which may turn into blue or purple denoted positive result.

Test for the Presence of Tannins: A 0.25 g of extract was boiled in 5.0 mL distilled water and was filtered. Few drops of 0.1% FeCl₃ were added to the filtrate. Occurrence of brownish green color indicated positive result.

GC-MS Analysis: A 1.0 mg of the crude leaf extract was diluted with a mixture of 0.5 mL absolute methanol and 0.5 mL dichloromethane to separate chlorophyll. The upper layer with no chlorophyll (10.0 µL) was then taken and further diluted with 1.0 mL chloroform.

The GC-MS analysis was carried out using agilent technologies 7890A GC system coupled to a mass detector, 5975C mass selective detector, and driven by agilent chemstation software and with HP-5MS 30 m × 0.25 mm × 0.25 µm df capillary column. The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min. and a liner velocity of 37 cm/s. The temperature of the injector was set at 320 °C. The instrument was set to an initial temperature of 70°C which was programmed to increase to 280 °C at the rate of 10 °C / min with a hold time of 4 min. at each increment injection. An aliquot of 1.0 µL sample was injected in a split mode 100:1. The mass spectrometer was operated in the electron ionization mode at 70eV and electron multiplier voltage at 1859V.

Other MS operating parameters were: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 3 min and scan range 22 - 550 amu (automatic mass unit). The compounds were identified by direct comparison of the mass spectrum of the analyte at a particular retention time to that of a reference standard found in the National Institute of Standards and Technology (NIST) library. Total GC running time was 45 min³⁶.

Brine Shrimp Lethality Test (BSLT): To evaluate the cytotoxicity of the extracts, BSLT was performed. BSLT followed the methods described^{37, 38}.

Hatching of the Shrimp: Brine shrimp (*Artemia salina*) cysts were hydrated by tap water for many hours until they swell. Hydrated eggs were transferred to a container with dark and light compartments filled with filtered seawater and a pinch of sodium carbonate and were fully aerated. The cysts were added on the dark side. After 24 h, the nauplii were collected with the use of Pasteur pipette.

Preparation of Test Solution: There were four test concentrations with three replicates for each concentration. To make the stock solution, 30.0 mg of the extract were dissolved in 3.0 mL of ethanol for alcohol extract and filtered seawater for aqueous extract to produce a 10000.0 µg/mL. Using a micropipette, 500.0 µL, 250.0 µL, 50.0 µL and 5.0 µL of the stock solution were transferred to four test tubes to produce 10.0 µg/mL, 100.0 µg/mL, 500.0 µg/mL, and 1000.0 µg/mL concentrations upon dilution to 5.0 mL of sterile filtered seawater.

Five milliliters (5.0 mL) of filtered sterile seawater served as control. For the alcohol- based extracts 150.0 µL of DMSO was added for the concentrations 500.0 µg/mL and 1000.0 µg/mL while 75.0 µL of DMSO were added for the concentrations 10.0 µg/mL and 100.0 µg/mL.

Bioassay: Ten larvae of *A. salina* were placed in every test tube with filtered seawater. The tubes were kept uncovered under 100-watt illumination. The mortality rate was determined after 6 and 24 h and the mortality percentage was calculated with the following formula,

% mortality = (No. of dead nauplii/initial no. of live nauplii × 100)

Lethality concentration (LC₅₀) was assessed using probit analysis at 95% confidence intervals. LC₅₀ values < 1000.0 µg/mL is toxic while LC₅₀ values >1000 µg/mL is non-toxic³⁶.

Statistical Analysis: All experiments were carried out in three replicates. Data were expressed in Mean ± Standard Deviation. Statistical analysis was carried out with SPSS software (version 20.0). Difference at p<0.05 was considered statistically significant.

TABLE 1: ALPHA-AMYLASE INHIBITORY ACTIVITY OF *C. SPECIOSUS* LEAF EXTRACTS

Extracts	% Activity			
	200 µg/mL	400 µg/mL	800 µg/mL	IC ₅₀ µg/mL
70% ethanol	68.77 ± 1.28	71.76 ± 0.90	80.73 ± 0.47	<200.0
aqueous	42.36 ± 0.83	54.65 ± 2.03	79.07 ± 0.47	288.12
Acarbose*	71.26 ± 1.04	76.41 ± 0.72	81.73 ± 0.85	<200.0

*standard

Moreover, the IC₅₀ of the 70% ethanol and aqueous extract is more or less the same with the acarbose with values <200.0 µg/mL and 288.12 µg/mL. Thereby, the extracts may be considered potent alpha-amylase inhibitors. This might be due to the capacity of the extracts to inactivate the alpha-amylase therefore binding to the active site of the enzyme and thereby blocking the conversion or cleavage of starch molecules into 1, 4 glycosidic linkage^{39, 40, 41}. More importantly, in the presence of inhibitors, the digestion of the final products of carbohydrates occurs slower in the intestine resulting to slower absorption of monosaccharides hence, results in the decrease of postprandial glucose produced^{2, 42}.

The assay performed was dose- dependent wherein the activity increased as the concentration was increased **Fig. 1**. This is supported by the study of Satyanaranan *et al.*,³⁹ wherein the alcohol extract showed a dose dependent manner in the inhibition of the alpha-amylase activity by the leaf of *Costus igneus*.

TABLE 2: MEAN DPPH SCAVENGING ACTIVITY OF HYDROALCOHOL AND AQUEOUS LEAF EXTRACTS OF *C. SPECIOSUS* AT DIFFERENT CONCENTRATIONS

Extracts	% Activity				
	0.25 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	IC ₅₀ mg/mL
70% ethanol	17.04 ± 0.16	37.82 ± 0.63	70.25 ± 0.86	78.03 ± 1.61	0.69
aqueous	29.3 ± 0.21	42.6 ± 0.26	42.75 ± 0.46	52.02 ± 0.02	1.65
Ascorbic acid*	65.17 ± 3.95	72.20 ± 0.39	73.39 ± 0.30	83.86 ± 1.32	<0.25

*standard

RESULTS AND DISCUSSION:

Alpha-Amylase Inhibitory Activity: The standard acarbose exhibited alpha-amylase inhibitory activity from 71.26 ± 1.04 to 81.73 ± 0.85 µg/mL at a concentration of 200.0 - 800.0 µg/mL, respectively, with an IC₅₀ value of <200.0 µg/mL **Table 1**. The hydroethanolic leaf extract showed higher inhibitory activity compared to the aqueous extract at concentration 200.0 µg/mL to 800.0 µg/mL with values from 68.77 ± 1.28 to 80.73 ± 0.47 µg/mL and 42.36 ± 0.83 to 79.07 ± 0.47 µg/mL with an IC₅₀ value of <200 µg/mL and 288.12 respectively.

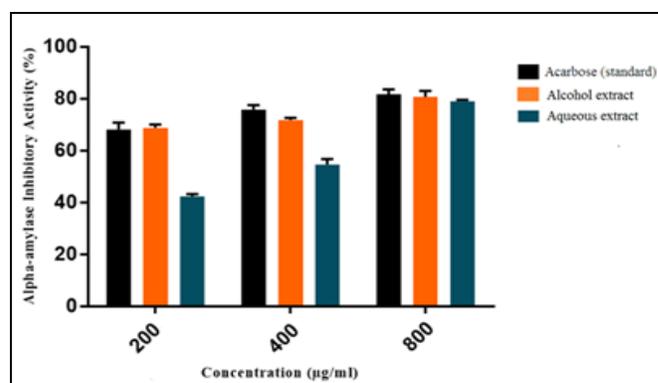


FIG. 1: ALPHA-AMYLASE INHIBITION OF 70% ETHANOL AND AQUEOUS EXTRACTS OF *C. SPECIOSUS* LEAF

DPPH Radical Scavenging Activity: **Table 2** shows the mean DPPH scavenging activity of hydroalcohol and aqueous leaf extracts of *C. speciosus* at different concentrations. The 70% ethanol has higher activity compared to aqueous extract and is more or less the same with the standard ascorbic acid with values 78.03 ± 1.61 mg/mL and 83.86 ± 1.32 and IC₅₀ values of 0.69 mg/mL and 1.65 mg/mL, respectively.

The antioxidant potential of the 70% ethanol might contribute to the alpha-amylase inhibitory activity where it also has higher alpha-amylase inhibition activity compared to the aqueous extract. Thus, antioxidant activity significantly affects the alpha-amylase inhibitory activity of the leaf extract ($r = 0.988$). The role of oxidative stress in diabetes and diabetic complications has been reported^{41, 42}. Oxidative stress is an important risk factor for diabetes, cardiovascular, oncologic, and many other diseases. Due to glucose oxidation and subsequent oxidative degradation, free radicals are formed disproportionately in diabetes⁴¹.

Total Phenolics Content: The total phenolics content of the leaf extracts of *C. speciosus* was found to be very low as shown in **Table 3**. This might be due to the reagent used in the quantification of total phenolics which is the Folin-Ciocalteu reagent. Depending on the number of phenolic groups, phenolic compounds react differently with the Folin-Ciocalteu reagent⁴³. Moreover, the low total phenolics content might be due to the lack of specificity of the Folin-Ciocalteu reagent since it cannot differentiate between phenol type and non-phenolic compounds giving an inaccurate TPC^{44, 45}.

TABLE 3: TOTAL PHENOLICS CONTENT OF EXTRACTS EXPRESSED IN MEAN GALLIC ACID EQUIVALENCE (GAE)

Sample	Extracts	GAE (mg/g extract)
<i>C. speciosus</i>	70% ethanol	13.46 ± 0.025
	aqueous	6.10 ± 0.009

Phenols largely contribute to the antioxidant activity of a plant⁴⁶. However, the results indicate that 70% ethanol and aqueous leaf extracts possess low total phenolics content. Thus, the study suggests that the antioxidant activity of *C. speciosus* is not limited to phenolics only. Moreover, Wongsu et al.,⁴⁷ stated that the antioxidant activity of a plant may be also due to other antioxidant secondary metabolites present in the plant.

Phytochemical Analysis: Since time immemorial, plant-derived compounds are well known for their therapeutic values. The phytochemical constituents of a plant contribute to its therapeutic activity⁴⁸. Analysis on the compounds is very useful in the evaluation of some active biological components of medicinal plants. **Table 4** shows the qualitative

analysis of the ethanol leaf extract of *C. speciosus* which confirms the presence of alkaloids, flavonoids, phenols, steroids, tannins, and terpenoids.

TABLE 4: PHYTOCHEMICAL SCREENING ON THE LEAF EXTRACT OF *C. SPECIOSUS*

Phytochemicals	<i>C. speciosus</i>
Alkaloids	++
Flavonoids	+++
Phenols	+
Saponins	-
Steroids	++
Tannins	++
Terpenoids	+++

*+++ (Intense color/ heavy precipitate)

++ (Moderate amount)

+ (Turbid only/ very light color)

- (Negative/ below the detection limit of the method used).

GC- MS Analysis of Leaf Extract: The components present in the ethanol extracts of *C. speciosus* were identified by GC-MS as shown in **Table 5**.

TABLE 5: PHYTOCHEMICAL COMPOUNDS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *C. SPECIOSUS* LEAVES

Compound	Similarity Index* (%)	Abundance* (%)
D-Limonene	94	5.19
Phenol, 2, 5-bis (1,1-dimethylethyl)	96	6.34
Eicosane	72	1.64
7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6-9-diene-2,8-dione	96	3.41
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	86	2.71
Hexadecanoic acid, ethyl ester	98	3.66
Isopropyl palmitate	86	1.03
Octadecanoic acid, ethyl ester	97	1.65
Hexadecanoic acid, bis (2-ethylhexyl) ester	87	3.78
Bis (2-ethylhexyl) phthalate	80	3.16
Nonadecane	89	3.12
Octacosane	91	1.62
Squalene	99	8.17
Tetradecane	86	1.80

All compounds given are identified by name as they showed more than 80% similarity when compared to the reference library. There were 14 compounds detected in the 70% ethanol extract of *C. speciosus*. The major compounds are squalene (8.17%); phenol, 2, 4-bis (1, 1-dimethylethyl) (6.34%); d-limonene (5.19%); hexadecanoic acid, bis (2-ethylhexyl) ester (3.78%); hexadecanoic acid, ethyl ester (3.66%); 7, 9-Di-tert-butyl-1-oxaspiro (4,5), deca-6-9-diene-2, 8-dione (3.41%) bis (2-

ethylhexyl) phthalate (3.16%); nonadecane (3.12%); and 1, 2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (2.71%). Other compounds which are present in lesser amount are eicosane, isopropyl palmitate, octacosane, and tetradecane. The presence of these active components reveals the importance of plants as medicinally used. Eicosane and nonadecane have both antioxidant and antidiabetic potentials⁴⁹. Squalene⁵⁰, phenol, 2, 4-bis (1, 1-dimethylethyl)⁵¹, D-limonene⁵², and hexadecanoic acid, ethyl ester^{53, 54} possess antioxidant potential.

Brine Shrimp Lethality Test: The cytotoxicity activity of the 70% ethanol and aqueous leaf extract

of *C. speciosus* is summarized in **Table 6**. The percentage mortality of the extracts increased as the concentration was increased. The LC₅₀ of the 70% ethanol extract is 730.92 µg/mL denoting moderate toxicity of the extract since LC₅₀ value < 1000 µg/mL is toxic while LC₅₀ value >1000 µg/mL is non-toxic³⁷.

This might be due to some cytotoxic compounds present in the extract. On the other hand, the aqueous extract has an LC₅₀ of 1,997.4 µg/mL which simply implies that this extract is safe to use and thus supports the traditional use of *C. speciosus* leaves prepared and taken through decoction.

TABLE 6: BRINE SHRIMP LETHALITY TEST OF 70% ETHANOL AND AQUEOUS LEAF EXTRACT OF *C. SPECIOSUS*

Extracts	% Mortality at Different Concentrations*				LC ₅₀ , 24h µg/mL
	10 µg/mL	100 µg/mL	500 µg/mL	1000 µg/mL	
70% ethanol	0	33.33	56.67	60.00	730.92
aqueous	0	33.33	6.67	13.33	>1000

*mean (n = 3)

CONCLUSION: The result suggests that the extracts exhibited alpha-amylase and antioxidant activity confirming the ability of the leaves of *C. speciosus* in the mitigation of postprandial hyperglycemia and may be a natural source of alpha-amylase inhibitor agent in combating diabetic complications. The negative correlation of the antidiabetic and antioxidant activities with the total phenolics content implies that these activities are not only limited to phenolics but also due to other secondary metabolites present in the leaf extracts such as eicosane, nonadecane, squalene, phenol, 2, 4-bis(1, 1-dimethylethyl), D-limonene, and hexadecanoic acid, ethyl ester that were identified through GC-MS.

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