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Chemical composition, *In vitro* antioxidant and anti-diabetic potentials of ethanolic extract of *Syzygium aromaticum* bud

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Abstract
Syzygium aromaticum bud is a functional food possessing a broad spectrum of biological activities attributable to its chemical compounds found within the plant. The aim of this paper was to conduct an investigation on chemical composition, *in vitro* antidiabetic and antioxidant potentials of ethanolic extract of *Syzygium aromaticum* bud. Gas chromatography-mass spectrometry (GC–MS) analysis was carried out to ascertain bioactive components of extract and antioxidant potentials were assayed via 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*) radicals scavenging activities and antidiabetic potential were investigated via two inhibitory enzymes such as α-amylase and α- glucosidase using spectrophotometer. This present study revealed percentage yield extract of 19.83%. In total, 6 components were identified and quantified such as 3-Ally-6-methoxyphenol (26.52%) which was the major compound followed by eugenol (22.44%) and *p* –amidino benzamide (20.40%). It was shown that the extract has IC50 value of 72.42 µg/ml for DPPH and 35.26 µg/ml for ABTS* radicals scavenging activity which has a strong antioxidant and was able to compete with standard drug (vitamin c) with IC50 value of 25.34 µg/ml. The extract inhibited in a dose-dependent pattern. Notwithstanding, α- glucosidase (IC50= 20.81 µg/ml) showed higher inhibitory activity than α-amylase (IC50= 255.76 µg/ml) and this study revealed that *Syzygium aromaticum* bud contain eugenol which exhibited an antioxidant and antidiabetic potentials.

Keywords: *Syzygium aromaticum*, functional foods, antioxidant, antidiabetic

Introduction
Diabetes mellitus (DM) means a disorder in metabolism characterized by the body's inability to make or use insulin. Deficiency in insulin can bring about a chronic hyperglycemia and abnormalities in metabolic activities as reported by Oboh *et al*. (2015) [20]. Diabetes has become much more common over the world in the last two decades, rising from an estimated 30 million cases in 1985 to 425 million in 2017. As the prevalence of unhealthy behaviors, physical inactivity and obesity rises, incidence rates are estimated to rise to 629 million people by 2045 (American Diabetes Association, 2013) [6]. This gives the reason for a reasonably large amount of healthcare expenditure, underlining the necessity for better and less expensive treatment options. Regardless of the diverse medications and strategies that exist to manage diabetes, these are very expensive for large diabetic population in underdeveloped nations, not to mention their inherent side effects. As a result, new low-cost alternatives must be sought to address this critical health issue (Park, 2011) [21]. *In vivo* study regarding diabetes have provided evidence of excessive production of reactive oxygen species (ROS) and depleted level of antioxidant as reported by Adisa *et al*. (2012) [5] that leads to disorganize cellular organelles and enzymes damage. *Syzygium aromaticum* (clove) is a member of Myrtaceae family that originated in the Islands of Maluku located in eastern Indonesia (Cortes-Rojas *et al*., 2014) [9]. It grows to a height of 12 meters, with the most well-known feature being the flower buds that appear four years after planting and are harvested before flowering (Kamatou *et al*., 2012) [16]. Clove buds possess a characteristic pungent odour and are generally dark-brown in colour. They are traditionally used when processed (in combination with cereals, legumes, and some kinds of fruits and vegetables) to produce a powdery substance. They are also used as whole spices (Adefegha and Oboh, 2013) [13]. Baghshahi *et al*. (2014) [7] reported that *Syzygium aromaticum* is an

effective herbal antioxidant that contains phenolic compounds mainly eugenol, >50%. In addition, Kabuto *et al*. (2007) [15] found that eugenol scavenges free radicals while increasing glutathione and L-ascorbate in mouse cells. This study was conducted to evaluate chemical composition, *in vitro* anti- diabetic and antioxidant potentials of the ethanolic extract of *Syzygium aromaticum* bud.

Material and Method

Collection and Identification of Sample

Dried clove bud (*Syzygium aromaticum*) was obtained from Oja-oba, Ilorin, Kwara State. The plant was taxonomically identified and authenticated in University of Ilorin with voucher specimen (UILH/001/1107/2021) which was deposited in the herbarium for future reference.

Preparation and Extraction of Sample

The air-dried and pulverized buds of *Syzygium aromaticum* were extracted in 70% ethanol for three (3) days at 370C, after which the extract was filtered, and concentrated using an oven at 450C. Percentage yield of plant extract was estimated employing the equation below

$$percentageyeild = \frac{weight\ of product}{weight of starting material} X100\%$$

GC-MS analysis

The analysis of the extract was done using an Agilent-7890A GC apparatus with a mass spectrometry as detector, as described by Momoh *et al*. (2019) [19]. The National Institute of Standards and Technology MS library database was used to detect the component (NIST-MS library) via comparison of retention indices.

Estimation of DPPH Scavenging Ability of extract

The free radical scavenging ability of ethanol-based plant extract against DPPH* was assessed using the method reported by Chan *et al*. (2007) [8] with slight modification.
Briefly, 1 ml of appropriate dilutions of the extract (10,20,50,100, and 150 g/ml) were mixed with 3 ml of 60 µM ethanolic solution of DPPH radicals and left in dark room for 30 minutes, after which the absorbance was determined at 517 nm. This was computed with the aid of the formular below;

% Inhibition= [(A0−A1)/A0] ×100

Where A0 is the absorbance of the control solution, A1 is the absorbance of sample or standard

Estimation of ABTS*+ scavenging assay

The intrinsic ability of the extract to scavenge ABTS*+was accomplished following the method revealed by Re *et al*. (1999) [25]. ABTS* working reagent was made ready by completely reacting equivalent volume of aqeous solutions of ABTS*+ (7millimoles/L), K2S2O8 (2.45millimoles/L) and incubate in dark at room temperature for 16 hours. Subsquently, the absorbance was measured and adjusted to 0.70 ± 0.02 with ethanol (95%) at 734 nm.
Then, at that point, a reaction mixture comprising 2.0 ml of ABTS*+reagent was added to appropriate dilution of extract and incubated at 37 0C in dark room for period of 15 min. After this, the absorbance reading was taken (at 734 nm) in a UV-Visible spectrophotometer utilizing ascorbic acid as standard.

% Inhibition= [(A0−A1)/A0] ×100

In vitro alpha-amylase inhibition assay

The method depicted by Kwon *et al*. (2008) was used to carry out alpha- amylase inhibition assay. In this assay, a soluble starch substrate and porcine pancrease α-amylase (EC 3.2.1.1) were used. A total of 500 µl of ethanol extract of various dilution and 500µl of 0.02M sodium phosphate buffer (pH6.9 with 0.006M NaCl) consisting 0.5mg/mL α-amylase solution was incubated at 37°C for 10 minutes. Also, 500 µL of starch solution (1%) in 0.02M Na3PO4 buffer was added. Next, the mixture was exposed to incubation at room temperature for a period of 15 min, after this, 1.0 ml of DNSA color reagent (1% 3,5-dinitrosalicylic acid coupled with 12% sodium potassium tartrate in 0.4M NaOH) was introduced to terminate their action. Then, the mixture was allowed for incubation for 5min and diluted with 10 ml distilled water upon cooling to room temperature.
The absorbance was measured at 540 nm and percentage α-amylase inhibitory effect was estimated applying the average absorbance of three fold tests as shown Below:

% Inhibition= [(A0−A1)/A0] ×100

Where A0 is the absorbance of the control solution, A1 is the absorbance of sample.

In vitro alpha-glucosidase inhibition assay

Alpha- glucosidase inhibitory activity was conducted following the method described by Qaisar *et al*. (2014) using *Bacillus stearothermophillus* α-glucosidase (EC 3.2.1.20) and para-nitrophenyl glucopyranoside (*p*-NPG) as substrate. Five units aliquot of α-glucosidase was incubated with appropriate dilution of ethanolic extract for 15 min. Following this, 3mM *p*-NPGwas dissolved in 20 mM phosphate buffer (pH6.9) and introduced as a substrate to start up the hydrolytic reaction. This reaction was terminated by the addition of 2 millilitres (2ml) of 0.1M sodium carbonate; after it was allowed to continue for 20 minutes at 370C. Ayellow *p*-nitrophenol was released upon the hydrolysis of *p*-NPG, which absorbance was read at 405 nm. The percentage α-glucosidase inhibition was determined using the absorbance derived from triplicate test, as follows:

% Inhibition= [(A0−A1)/A0] ×100

Result and Discussion

The results in table 2 above present the chemical compositions of ethanol-based extraction products of *Syzygium aromaticum* bud as analyzed by GC-MS. A total of six (6) bioactive components were identified, which constitutes 99.99% of the entire amount. It is also revealed that 3-allyl-6-methoxyphenol (26.52%) and eugenol (22.44%) were identified to be the major components of extract, which has similar molecular mass but differ in structural arrangement. Similar to these findings, some research work reveal that eugenol forms the major component of the essential oilof clove, differing with respect to peak area or content, according to the reports by

Tang *et al*. (2008) [26]. According to this research, eugenol has a percentage content which differs from the previous findings by Hafizah *et al*. (2015) [12], concerning its essential oil. They reported eugenol (18.7%) as major component of S. aromaticum with lower content compare with present study. Oxidative stress is characterized by excessive free radical accumulation associated with many pathogenesis of diseases such as diabetes and obesity (Unuofin and Lebelo, 2020) [27]. Hence, compounds with antioxidant properties, harbor a significant potential to complement anti-diabetic treatment regimes. The methanolic extract of S. aromaticum bud showed strong antioxidant potentials via DPPH assay which was reflected by IC50value of 44 µg/ml compared with ethanolic extract of S. aromaticum bud with IC50value of 72.42 µg/ml as reported by Abd El Azim *et al*. (2014).This experimental study on ABTS scavaging activity in Figure 3 showed a powerful antioxidant potential with IC50= 35.26 µg/ml when compared with Xin and Jian (2017) [28] who reported the scavenging capacity of oleoresin from clove bud with IC50 value of 85.9 µg/ml.

Plant based products particularly the dried buds of ethanolic extract of *Syzygium aromaticum* was reported to involve in varieties of biological role.This study revealed that α-amylase and α-glucosidase inhibitory actions (displayed in Figure 4 and 5) followed a dose-dependent pattern. However, α-glucosidase (IC50= 20.81µg/ml) showed higher inhibitory activity than α-amylase (IC50= 255.76µg/ml) in this study, which agrees with already published works that particular phytochemicals are mild inhibitors of α-amylase and strong inhibitors of α-glucosidase activity (Ranilla *et al*., 2010; Oboh *et al*., 2010). This works are superior to a previous study, who examined poor inhibition of α-amylase activity by free phenol (IC50= 497.2 µg/ml) and bound phenol (IC50= 553.7µg/ml) extracts of S. aromaticum (Adefegha and Oboh, 2012) [4].

The presence of insulin mimetic agents in the extract might be responsible for its anti-diabetic activity (Kuroda *et al*., 2012; Prasad *et al*., 2005) [17, 5]. Hence, considering the mild and stronger inhibitory effect of the extract on α-amylase and α-glucosidase activities respectively. We infer that the extract is of tremendous neutraceutical relevance, and for cutailing concomitant adverse effects of the anti-diabetic drug (Acarbose). One of the mechanisms explored by clove bud (used in various traditional medicines) in managing diabetes might be by slowing down the hydrolysis of starch in the gastrointestinal tract.

In conclusion, result of this study clearly indicate that, anti-diabetic and antioxidant activities of dried flower bud of *Syzygium aromaticum* could be attributed to its phenolic (eugenol) content which was mediated via gastrointestinal starch inhibitory and free radical scavenging activity.

Table 1: Result of percentage yield of ethanolic extract of *Syzygium aromaticum* bud

S/n	Sample	Weight of Pulverized Sample (g)	Weight of Extract (g)	Colour	Percentage Yield (%)
1	*Syzygium aromaticum*	100	19.83	Dark brown	19.83

Table 2: Result of GC-MS analysis of the extract

Peak number	Retention time (RT)	Formular	Compound name	Molecular weight (MW)	Area (%)
1	3.175	C2H4S	Thiirane	60.12	3.85
2	4.476	C8H17F	1-fluorooctane	132.22	13.52
3	11.870	C10H12O	3-Ally-6-methoxyphenol	164.24	26.52
4	12.070	C8H9N3O	*p*–Amidinobenzamide	163.18	20.40
5	12.865	C10H12O2	Eugenol	164.20	22.44
6	14.091	C9H10N2O4	2-amino-5-ethyl-3-nitro-benzoic acid	210.19	13.26

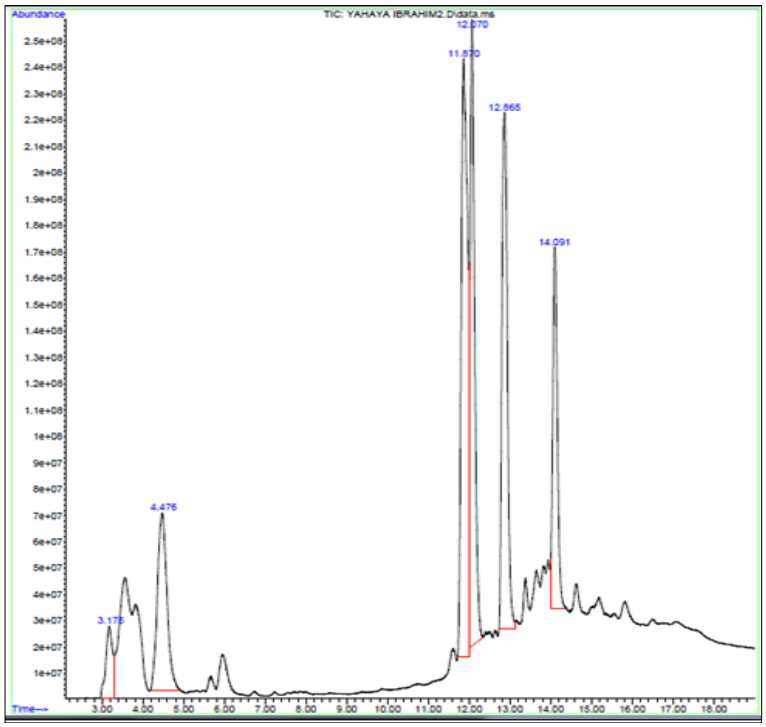

Fig 1: Chromatogram of the extract, showing retention time and peak area

Table 3: Result showing antioxidant potentials of ethanolic extract of *Syzygium aromaticum* bud

S. No	Sample(s)	DPPH (IC50 µg/mL)	ABTS (IC50 µg/mL)
1	*Syzygium aromaticum*	72.42	35.26
2	Standard drug (Vitamin C)	22.22	25.34

Data represent the mean of triplicate readings

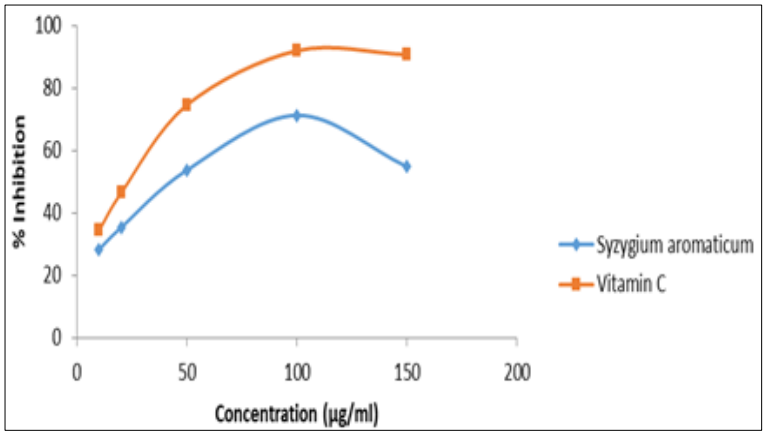

Fig 2: Graphical representation of DPPH radical scavenging activity of extract

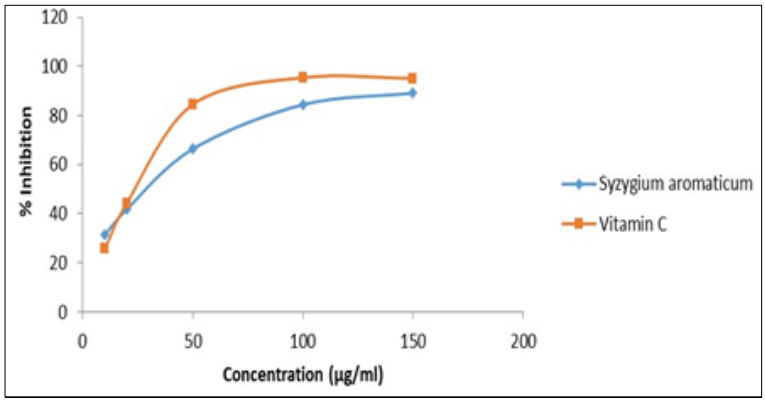

Fig 3: Graphical representation of ABTS. + scavenging activity of ethanolic extract

Table 4: Result of anti-diabetic potentials of ethanolic extract of *Syzygium aromaticum* bud

S/N	Sample	α-amylase (IC50	α-glucosidase (IC50
1	*Syzygium aromaticum*	255.76	20.81
2	Standard drug	33.06	1.17

Data represent the mean of triplicate readings

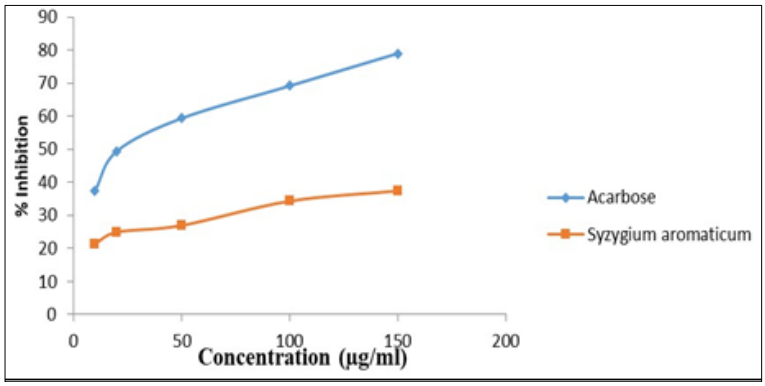

Fig 4: Graphical representation of alpha amylase inhibitory activity of the extract

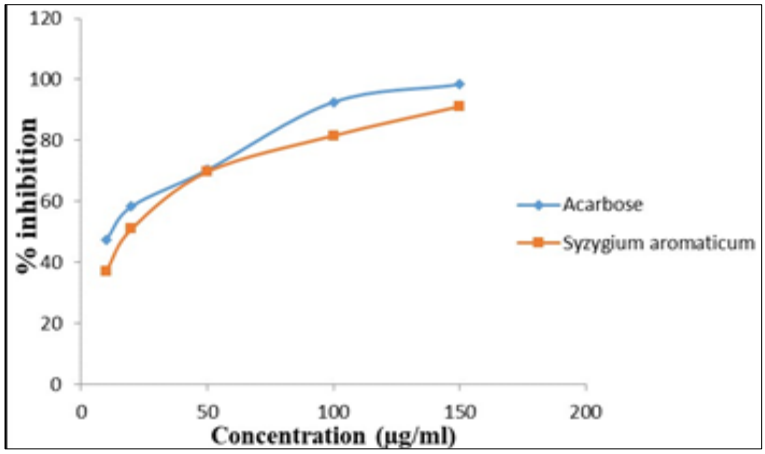

Fig 5: Graphical representation of alpha glucosidase inhibitory activity of ethanolic extract

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