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IN-VITRO PRELIMINARY PHYTOCHEMICAL ANALYSIS AND PHARMACOLOGICAL SCREENING FOR ANTIOXIDANT AND ANTIDIABETIC POTENTIALS OF *ORTHOSIPHON GLABRATUS* BENTH LEAF IN DIFFERENT SOLVENT FRACTIONS

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Orthosiphon glabratus leaves, DPPH, ABTS, Solvent fractions, Antidiabetic, Antioxidant, *α*-amylase, *α*-glucosidase

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ABSTRACT: In an attempt to reveal the medicinal values of *Orthosiphon glabratus* Benth leaf, which claims to have traditional use for the management of diabetes. *In-vitro* antioxidant and antidiabetic potentials of *Orthosiphon glabratus* leaf in different solvent fractions were studied by utilizing DPPH, ABTS, and *α*-amylase, *α*-glucosidase inhibitory methods respectively. A preliminary phytochemical study shows the presence of important constituents such as alkaloids, flavonoids, phenols, cardiac glycosides and terpenoids in all the solvent fractions. The ethyl acetate fraction exhibits the best inhibition activity against DPPH and ABTS, and its IC_{50} was found to be 2.28 and 1.68 µg/mL respectively, which is significantly lower ($P<0.05$) than reference. In case of antidiabetic activity, ethyl acetate fraction shows the highest enzyme inhibitory action against *α*-amylase and *α*-glucosidase and IC_{50} value was found to be 2.09 and 4.66 µg/mL respectively than ($P<0.05$) acarbose. The results of *in-vitro* antioxidant and antidiabetic assays on different solvent fractions of *Orthosiphon glabratus* leaf, it is evident that the ethyl acetate fraction could be potential candidature, which possessing the majority of phytochemical classes of compounds and it could be the reason for its antioxidant and antidiabetic activities. However, further studies are necessary to isolate the bioactive principles in *Orthosiphon glabratus* leaves that are responsible for management of diabetic and as antioxidant.

INTRODUCTION: Generally, the various environmental stresses are continuously exposed to the human body, as the results of this, the synthesis of highly reactive species, also called as free radicals (ROS/RNS) which causes the oxidation of cellular machinery by the electron-transfer reactions.

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All ROS are extremely harmful to organisms at high concentrations. For the purpose to encounter the damaging effects of such species, human bodies have endogenous antioxidant (defense) systems, and as well it obtains from outside antioxidants from the diet which have capable of neutralizing such species and maintains the homeostasis of the body. Any unbalance among the ROS, and endogenous antioxidants may cause ‘‘oxidative stress’’[1,] and it has supposed as a factor which plays a central role in the pathoetiology and progress of diabetes [2]. As such, under the diabetic condition, the increased levels of ROS will damage the pancreas and liver cells.

Most of the studies expose the deduction of oxidative stress in diabetes pathogenesis by the modification in enzymatic systems, peroxidation of lipids, defective glutathione metabolism and decreased vitamin C and E levels [1]. Oxidative stress plays an essential part in advance of diabetes complications, in both microvascular (stroke, neuropathy, retinopathy, and nephropathy) and cardiovascular [3]. Diabetes is chronic hyperglycemic diseases that occur when the pancreas does not produce enough insulin or defective in insulin action or both. If left untreated, it can bring out the long term complications for organs such as heart, liver, kidney, eyes, nerves and veins. Disruption in some of these organs can lead to dealth [4]. There are three main forms of diabetes: type 1, type 2 and gestational diabetes [5]. The number of adults aged over 18 years with diabetes has lifted from 108 million in 1980 to 422 million in 2014 [6]. Diabetes affects more or less 2.8% of world's population and is anticipated to increase upto 5.4% by the year 2025 [7, 8].

The World Health Organization predicts diabetic deaths occur before the age of 70 years will double between 2005 and 2030, and it is a seventh leading cause of death in the year 2030. [9] Diabetes is also characterized by marked postprandial hyper-glycemia (PPHG). The inhibition of intestinal glucose absorption is one of the therapeutic approaches to decreasing postprandial hyperglycemia is by modifying the activity of carbohydrate-hydrolyzing enzymes, such as *α*-glucosidase and *α*-amylase, in digestive organs. Postprandial hyperglycemia also leads to protein glycation and glucose oxidation, releasing nitrogen and oxygen reactive species.

Over the last few decades, the accountability of medicinal plants as a principal tool in the preservation of health and management of diseases has been realized with great concern. This may due to mostly, use of synthetic drug molecules for their inefficiency with harmful side effects [10], which are comparatively fewer in drugs of medicinal plant origin [11]. This may due to mostly, use of synthetic drug molecules that produce harmful side effects, which are comparatively less in drugs of plant origin. It was reported earlier studies that natural antioxidants could prevent the key enzymes *α*-amylase, *α*-glucosidase and control the post-prandial hyperglycemic conditions, which is one of the possible approaches to managing the diabetes mellitus [12] and many plant's antioxidants also have hypoglycemic effects, which may in additionally contribute to improved glycemia. Thousands of herbal plants were traditionally used to treat diabetes, though some of them only screened for its phytochemicals and pharmacological activity [13] minority studies reported on. In spite of rich in the traditional medicinal values, merely few activities were reported so far in *Orthosiphon glabratus* leaves.

The genus *Orthosiphon* Benth in tribe Ocimeae comprises 40 species, and it is distributed mainly in tropical and subtropical Asia including Southern Africa and Madagascar. The species usually occurs in grassland, forest margins or woodland. This genus was widely used in traditional medicine to prevent different diseases such as diabetes, kidney stone, hypertension, edema, hepatitis, rheumatism, and jaundice [14]. *Orthosiphon glabratus* Benth herbaceous shrub is one of the *Orthosiphon* genus and belongs to the family Lamiaceae. It is distributed in roadsides and rocky crevices from hills above 600 m particularly in the southern part of India (Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu). Traditionally, *Orthosiphon glabratus* is used to cure diarrhea, piles, cuts, wound [15], fever [16] and diabetes [17]. The *Orthosiphon stamineus* Benth have been found to possess antidiabetic activity, *Orthosiphon stamineus* & *Orthosiphon thymiflorus* has been found to posses the antioxidant activity. However, the leaf of *Orthosiphon glabratus* Benth has not been uncovered for its antioxidant activity and pharmacological investigations. Therefore, present study was carried out to explore the antioxidant and antidiabetic activity of the leaf extracts of *Orthosiphon glabratus* Benth. by *in-vitro* methods.

MATERIALS AND METHODS:

Chemicals and Reagents: All the chemicals and reagents used in this work were of analytical grade. 1. 1-diphenyl -2 - picrylhydrazyl (DPPH) was purchased from Sigma (Sigma Aldrich Ltd., Mumbai, India), ascorbic acid and *α*-amylase were procured from Loba Chemie Pvt. Ltd., (Mumbai, India), 2, 2´-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) - (ABTS), potassium persulfate, Di-nitro salicylic acid, potassium phosphate buffer and

phosphate buffer saline were received from SRL (Sisco Research Laboratories Pvt. Ltd., Mumbai, India). Acarbose and p-nitro phenyl glucopyranoside were obtained from TCI Chemicals Pvt. Ltd., Chennai, India, *α*-glucosidase was purchased from SD Fine-Chem Ltd., Chennai, India. Ethanol, chloroform, ethyl acetate, and methanol were acquired from Fischer Chemic Ltd., Chennai, India.

Extraction and Fractionation: *Orthosiphon glabratus* leaves were collected from Meghamalai (Highways Mountains) of Tamil Nadu, India in December 2015 and authenticated by Dr. S. Soosairaj, Department of Botany, St. Joseph's college, Tiruchirupalli, Tamil Nadu. A voucher specimen of the plant is preserved in the herbarium of same institute for future reference (Herbarium no: SJCBOT2225). The leaves were washed with clean water and air-dried for 2 weeks and ground into coarse powder (500 g) was extracted with a 90:10 (v/v) mixture of ethanol and water using soxhlet apparatus. The resulting solvent was evaporated by rotary evaporator and dried over a water bath at a 45 ºC temperature to yield the hydroalcoholic extract of *Orthosiphon glabratus*. The dried extract was further fractionated with different solvents such as chloroform, ethyl acetate, and methanol. All the fractions obtained were further utilized for testing phytochemicals study, *in-vitro* antioxidant and antidiabetic activity.

Preliminary Phytochemical Study: Preliminary phytochemical analysis was carried out using standard methods described in the literature [18]. Samples of different fractions of *Orthosiphon glabratus* were tested for the presence of alkaloids, saponins, tannins, glycosides, flavonoids, phenols, steroids, terpenoids, quinones and proteins. Results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

***In-vitro* Antioxidant Assay:**

DPPH* Radical Scavenging Assay: The antioxidant activity of the different fractions was determined concerning radical scavenging ability or hydrogen donating capability using the stable radical 2,2-diphenyl-2-picrylhydrazyl (DPPH*) [19]. The solution of 0.135mM DPPH* was prepared in methanol. Different concentration of extract (0.5 ml) was mixed with 2.5 ml of DPPH* solution. The reaction mixture was vortexed thoroughly and left for 30 min at the dark room. The absorbance of the mixture was measured at 517 nm. Solution without sample was served as control and methanol served as blank.

Triplicate determination was made at each dilution of the sample, and the percentage inhibition of free radical scavenging capacity of the sample was calculated and expressed as IC_{50} values of the sample, *i.e*., the concentration of an inhibitor where the response of DPPH* is reduced by half. Ascorbic acid was used as the reference drug. The ability of plant extract to scavenge DPPH* radical was calculated from the following formula:

% DPPH inhibition = OD of control – OD of test / OD of control × 100

ABTS Cation Radical Scavenging Activity: ABTS cation radial activity was performed by the procedure, which described by Hua Fan *et al*. [20] A stock solution of ABTS radical cation was prepared by dissolving ABTS (7 mM, 25 ml in deionized water) with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). The mixture was left to stand for 15-16 h in the darkroom (the time required for formation of the radical) before use. The working solution was prepared by the previous solution and diluting it in ethanol to get the absorbance of 0.700 ± 0.02 at 734 nm. The solvent fractions (0.1 ml) at different concentrations were mixed with the ABTS working solution (1.9 ml), and the reaction mixture was allowed to stand at room temperature for 20 min, then the absorbance was measured by using a UV-Visible spectrophotometer at 734 nm. Ascorbic acid was used as the reference drug. The percentage inhibition for radical scavenging activity of is calculated by the equation given below, and the results of IC_{50} were expressed as the mean ± SD of three replicates.

% ABTS cation radial scavenging = OD of control – OD of test / OD of control × 100

***In-vitro* Antidiabetic Assay:**

***α*-amylase Inhibitory Assay:** *α*-amylase inhibitory activity was carried out by using the method described by Quangin Fei *et al*., [21] *α*-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25

ml) were mixed with an *α*-amylase solution (0.25 ml) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.5 ml 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 0.5 ml DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100 °C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. Acarbose was used as positive control. The result of triplicate determinations of *α*-amylase inhibitory activity was done and expressed as percentage inhibition by the following equation:

$$\text{Inhibition \%} = \text{Abs 1- Abs 2 / Abs 1} \times 100$$

The concentration of the sample causing 50% inhibition (IC_{50}) of α-amylase was calculated from its standard calibration curve.

***α*-glucosidase Inhibitory Assay:** The ability to inhibit *α*-glucosidase enzyme was studied by the method reported by Ju-Sung Kim *et al*. [22] The samples were reconstituted with distilled water and DMSO, respectively, at various concentrations. 450 µl of Extracts were incubated with *α*-glucosidase (50 µl, 0.5 Units/mL) and 0.2M potassium phosphate buffer (1500 µl, pH 6.8) at 37 ºC in a water bath for 15 min. Then, 250 µl of 3mM p-nitrophenyl glucopyranoside (PNPG) was added as substrate. The reaction was incubated again for ten minutes and then stopped by the addition of 750 µl of 0.1M Na_2CO_3. The absorption of 4-nitrophenol, a product after the reaction, was measured at 405 nm using a UV-Vis spectrophotometer. Reaction mixture without the sample served as negative control and reaction mixture without the substrate served as blank. Acarbose was used as positive control. The percentage inhibition of *α*-glucosidase triplicate experiments was computed by the following equation and expressed as IC_{50} using standard calibration curve.

$$\alpha\text{-glucosidase inhibitory activity} = [(AC^{+} - AC^{-}) - (AS - AB)] / (AC^{+} - AC^{-}) \times 100$$

Statistical Analysis: Results of triplicate determinations were expressed as mean ± SD. Statistical analysis was done by using the graph pad prism software. Statistical difference between means of test sample and control sample were evaluated by one way ANOVA followed by turkey's multiple comparison tests.

RESULTS AND DISCUSSION:

Phytochemical Screening: Secondary metabolites such as phenols, alkaloids, glycosides, flavonoids, terpenoids, tannins and carotenoids of the plant reported to possess a wide range of various physiological and pharmacological effects on human body [23] and as well natural plants have long been used to cure diabetes, as for their principle phytochemical components showed good anti-diabetic and antioxidant properties [24].

In our study, from the result of qualitative phytochemical analysis with the different solvent fractions of *Orthosiphon glabratus* leaves, it showed the presence of secondary metabolites, cardiac glycosides, alkaloids, flavonoids, phenols and terpenoids (shown in **Table 1**) in all the fractions. However, saponin was detected in chloroform and methanol fractions but absences in ethyl acetate fraction. In other hand, tannins were present in methanol and absences in chloroform and ethyl acetate fractions. Presence of steroids was identified in chloroform fraction except in ethyl acetate and methanol extracts. Moreover, quinones and proteins were absent in all the fractions. These findings confirm that *Orthosiphon glabratus* leaf contains the molecules know for extensive uses in the medicinal field both traditionally and pharmaceutically.

It was known the fact that phenols from plant sources are one of the key components which act as free radical terminators, anti-aging, anti-inflammatory, and anti-cancer [25, 26] and flavonoids have biological activities such as anti-inflammatory, antioxidant and anticancer [27, 28]. The various phytochemical of tannins, flavonoids, saponins, anthraquinones, terpenoids, phlorotannins and polyphenols existence in the plant may have the capability to inhibit the α-amylase and *α*-glucosidase [29, 30]. Alkaloids were also documented for its anti-diabetic and antioxidant potentials [31]. Thus, from the preliminary phytochemical screening test, this suggested having their antidiabetic and antioxidant activity of *Orthosiphon glabratus* leaves, owing to the presence of phytochemicals.

TABLE 1: SHOWS THE PHYTOCHEMICAL CONSTITUENTS PRESENCE IN DIFFERENT SOLVENT FRACTIONS *OF ORTHOSIPHON GLABRATUS* LEAF

Tests for	Solvent fractions		
	OG-$CHCl_3$	OG-EA	OG-Met.OH
Alkaloids	+	+	+
Saponins	+	-	+
Tannins	-	-	+
Flavonoids	+	+	+
Phenols	+	+	+
Cardial glycosides	+	+	+
Steroids	+	-	-
Terpenoids	+	+	+
Quinones	-	-	-
Protein	-	-	-

In-vitro Antioxidant Assay:

Free Radicals Scavenging Activity: Free radical scavengers are significant regarding the oxidative stress which would be affected and damage biotic molecules [32]. Owed to undesirable side effects of certain commercially available antioxidant, the natural resources of plant antioxidant with its well known healthy benefits have emerging in recent years [33]. There are enormous phytoconstituents with an antioxidant activity which play an important role in ceasing the production of a free radical chain reaction. Many researchers have shown that natural resources of antioxidants in plants closely related to its biological functions such as suppression and prevention of ageing and many chronic oxidatitive related diseases like cancer, diabetes, cardiovascular disease, rheumatoid arthritis, autoimmune disease and AIDS [34].

Hence, in this study, evaluation of anti-radical scavenging properties of a different solvent fraction of *Orthosiphon glabratus* leaves for a new antioxidant by utilizing DPPH and ABTS radical scavenging assays. A single assay to determine the antioxidant property would not give the correct result owing to influenced by many factors, for example, the test system and composition of the different solvent fractions.

In DPPH assay, the highest percentage of scavenging activity was obtained from methanol fraction (98.32%), ethyl acetate fraction (98.05 %) **Table 2** than the ethyl acetate fraction (98.05 %) and chloroform fraction (95.78%). In case of ABTS assay, the percentage scavenging activity of all fractions **Table 3** from *Orthosiphon glabratus* is presented in descending order, ethyl acetate fraction > methanol fraction > chloroform fraction.

TABLE 2: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS* LEAF

Concentration (µg/mL)	Ascorbic acid	DPPH % Inhibition		
		OG-$CHCl_3$	OG-EA	OG-MetOH
5	27.97	22.24	51.25	25.14
10	33.50	24.27	81.29	29.52
25	63.35	27.17	92.20	61.08
50	95.90	31.47	94.54	89.33
100	96.33	58.49	96.85	91.40
200	96.93	89.85	97.61	92.67
400	97.77	92.43	97.05	97.13
800	98.48	95.78	98.05	98.32

TABLE 3: ABTS RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS*

Concentration (µg/mL)	Ascorbic acid	ABTS % Inhibition		
		OG-$CHCl_3$	OG-EA	OG-Met.OH
5	32.83	18.88	64.11	56.85
10	65.74	44.24	86.82	68.63
25	86.50	50.85	90.66	84.50
50	95.39	62.27	91.59	87.64
100	95.88	83.23	92.90	88.94
200	96.90	86.21	93.59	90.90
400	97.75	88.41	95.63	91.72
800	98.61	91.10	98.16	93.84

From this estimation of the IC_{50} values of DPPH and ABTS with the different solvent fractions of *Orthosiphon glabratus* **Fig. 1** and **2**, the IC_{50} for ethyl acetate fraction was 2.28 µg/mL, showed significantly ($P<0.05$) excellent inhibition against DPPH, than other fractions and ascorbic acid.

However, in ABTS assay the ethyl acetate fraction exhibited significantly (P<0.05) greatest inhibitory effect (1.68 µg/mL), when compared only with chloroform fraction and ascorbic acid, but the methanol fraction of *Orthosiphon glabratus* exhibited highest scavenging ability followed by ethyl acetate fraction.

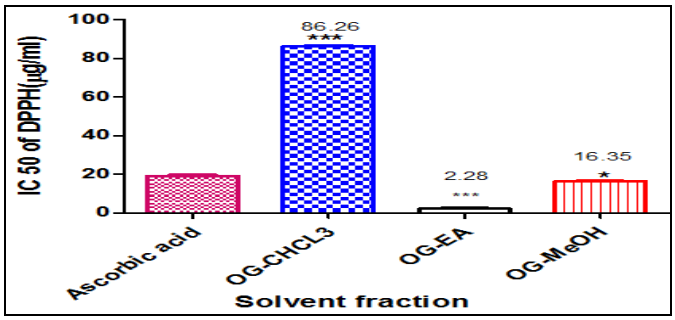

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS* LEAF

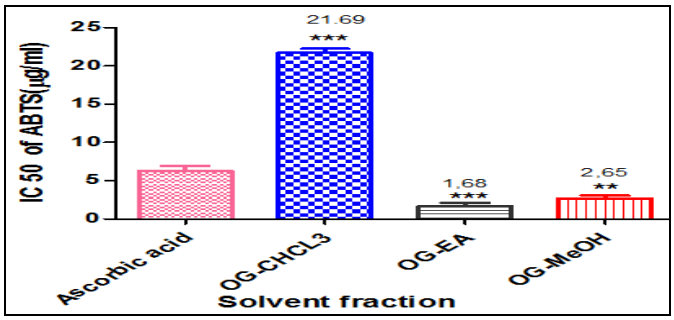

FIG. 2: ABTS RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS* LEAF

At this point, differences in antiradical activity of the fraction might depend on the polarity of the solvent and solute [35]. $DPPH^{\bullet}$ & $ABTS^{\bullet+}$ scavenging involves both hydrogen and electron transfer [36]. Thus the present investigation suggest that ethyl acetate fraction may contain enormous hydrogen donor molecule which could be the reason for the reduction in the production of free radicals and decolorization of DPPH and ABTS. The phytochemical study shows the presence of the phenolic compound, which was reported that it has an important role in stabilizing lipid peroxidation and direct antioxidant activity due to the presence of hydroxyl groups [37]. The antioxidant activity is not limited only to phenolic compounds, flavonoids also subgroups of phenol compounds which were reported to possess strong free radical scavenging based on the ability to act as electron or hydrogen donors and chelate transition of metals [38, 39]. Hence, the antioxidant activity of different solvent fractions of *Orthosiphon glabratus* might be exerted by their presence of phytoconstituents like phenols and flavonoids.

***In-vitro* Antidiabetic Assay:** Diabetes is also characterized by postprandial hyperglycemia [40]. The α-amylase and α-glucosidase are the two enzymes, which hydrolysis the starch into sugars and disaccharides leading increase the level of glucose level [41]. Reducing raised blood sugar and subsequent complication is also one of the treatment in controlling diabetes, treatment with oral hypoglycemic agents such as *α*-amylase and *α*-glucosidase inhibitors causes various side effects such as flatulence, abdominal distention and diarrhea [42], thus searching of inhibitors from natural sources has emerged in recent years.

The inhibitory effect of different solvent fractions of *Orthosiphon glabratus* and acarbose on α-amylase and *α*-glucosidase are shown in **Table 4** and **5**. The highest percentage inhibition of different solvent fractions of *Orthosiphon glabratus* against *α*-amylase was shown in the range of 83.16% to 87.58%, which was higher inhibitory activities compared to reference 64.03%, whereas, against α-glucosidase, the highest percent of inhibition of all fractions were ranged from 40.28% to 69.90%, when it's been compared to standard acarbose 95.85% those were show lesser inhibitory activity.

TABLE 4: *α*-AMYLASE RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS*

Concentration (µg/mL)	*α*-amylase % Inhibition			
	Acarbose	OG-$CHCl_3$	OG-EA	OG-Met.OH
50	2.11	13.40	34.11	21.25
100	9.44	27.56	41.29	33.91
250	18.71	37.74	58.37	43.64
500	36.67	48.86	74.52	66.95
1000	56.28	64.96	81.53	75.82
2000	64.03	83.16	85.33	87.58

TABLE 5: *α*-GLUCOSIDASE RADICAL SCAVENGING ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS*

Concentration (µg/mL)	*α*-glucosidase % Inhibition			
	Acarbose	OG-$CHCl_3$	OG-EA	OG-Met.OH
5	5.57	2.547	3.82	2.07
10	24.84	6.84	9.39	4.77
20	42.83	20.85	24.84	10.19
40	57.96	24.84	38.69	19.90
80	83.59	27.70	53.50	22.61
160	94.90	33.28	66.71	33.59
320	95.85	36.94	69.90	40.28

The IC_{50} value for inhibitory efficiency of different solvent fractions of *Orthosiphon glabratus* against α-amylase and *α*-glucosidase are presented in **Fig. 3** and **4**. Ethyl acetate fraction shows the best inhibition against α-amylase IC_{50} (2.09 µg/mL), significantly lower (P<0.05) than other fractions and significantly higher (P<0.05) than acarbose as well. Whereas, in the *α*-glucosidase inhibitory assay, the ethyl acetate fraction showed good inhibition and IC_{50} (4.66 µg/mL), which is significantly lower (P<0.05) than chloroform and methanol fractions and shows no significant (P<0.05) when compared to acarbose. Therefore, at this point, it evident that fraction of *Orthosiphon glabratus* could be valuable in the management of diabetes by delaying starch hydrolysis in GI tract, which in turn to cause a decrease absorption of glucose and consequently suppress the postprandial hyperglycemia [43]. This dual inhibitory potential against key enzymes might be due to the presence of bioactive compounds such as phenols and flavonoids [44, 45]. It is also reported that plants with phenols, flavonoids, alkaloids, glycosides, and terpenoids have antidiabetic potential [46]. In this study the ethyl acetate fraction showed better activity among other fractions and presence of phytochemicals in *Orthosiphon glabratus* could act synergistically to produce the observed anti-diabetic effect, this may also be related to the high antioxidant activity of the plant, thereby mopping up free radicals that could be produced under diabetic condition.

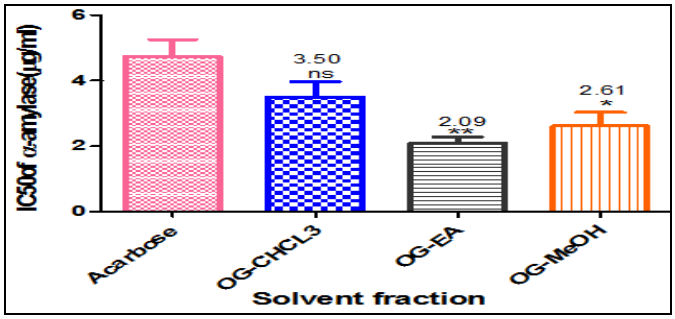

FIG. 3: *α*-AMYLASE INHIBITORY ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS* LEAVES

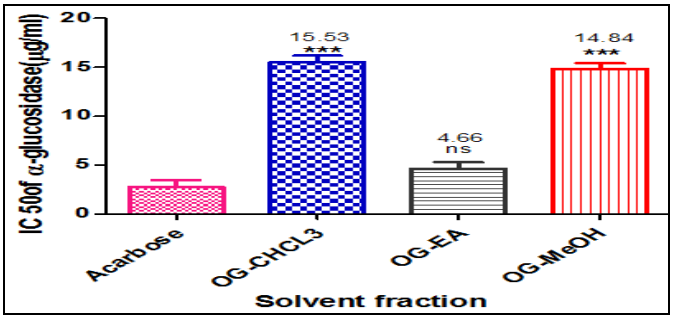

FIG. 4: *α*-GLUCOSIDASE INHIBITORY ACTIVITY OF DIFFERENT SOLVENT FRACTION OF *ORTHOSIPHON GLABRATUS* LEAVES

CONCLUSION: From the results of *in-vitro* antioxidant and antidiabetic assays on different solvent fractions of *Orthosiphon glabratus* leaves, it is concluded that the ethyl acetate fraction of *Orthosiphon glabratus* could be good candidature because it is possessing the majority of phytochemical classes of compounds and it could be the reason for its antioxidant and antidiabetic activities. However, further, with the aid of sophisticated animal studies are required to confirm and to isolate the bioactive principle in *Orthosiphon glabratus* leaves that are responsible for management of diabetic and as antioxidant.

CONFLICT OF INTEREST: The authors hereby declare no conflict of interest in the publication of this paper.

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